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PROGRESSIVE CHANGES IN THE WAXLIKE COATING ON THE SURFACE OF THE APPLE DURING GROWTH AND STORAGE[1]

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INTRODUCTION

In a previous communication one of the writers [2] reported the isolation and identification of the three principal constituents of the ether-soluble waxlike coating present on the surface of the apple. The investigation has now been extended to include a study of the progressive changes in this coating during growth and storage of the fruit. This phase of the work was prompted by questions arising in regard to the influence of the natural waxy coating of the fruit upon (1) the adhesiveness of sprays applied, (2) the removal of spray residues, and (3) the development of storage scald.

It is very probable that the chemical and physical properties of the surface-coating constituents and the relative quantities of these substances play an important rôle in the effectiveness of spray applications. Preliminary studies have shown that the percentage composition of the waxy coating of apples and therefore its physical properties change gradually throughout the growing season. A thorough study of the chemical and physical changes should, therefore, result in the accumulation of data which might be of considerable value in spray-research work.

It is recognized in certain sections of the country that the character of the surface covering of apples is of considerable importance in connection with methods of spray removal. In the Pacific Northwest a large percentage of the harvested apple crop is washed with chemicals to reduce the quantity of arsenic from spray materials to the amount permitted by law. Varieties such as Esopus Spitzenburg and Arkansas Black are more difficult to clean than others,[3] even when treated immediately after picking. Furthermore, any delay in cleaning these and certain other varieties results in increased difficulty in removing most of the spray residue. A possible explanation of this behavior is that waxlike substances may diffuse to the surface, particularly after the fruit is picked, and render the removal of the arsenicals more difficult. This theory is supported by the fact that such varieties feel greasier to the touch after standing and that oil sprays, especially when applied late in the growing season, make the fruit more difficult to clean later.

[1] Received for publication Nov. 29, 1930; issued June, 1931.

[2] SANDO, C. E. CONSTITUENTS OF THE WAX-LIKE COATING ON THE SURFACE OF THE APPLE. Jour. Biol. Chem. 56: 457–468. 1923.

[3] DIEHL, H. C., FISHER, D. F., HARTMAN, H., MAGNESS, J. R., and ROBINSON, R. H. REMOVAL OF SPRAY RESIDUE FROM APPLES AND PEARS IN THE PACIFIC NORTHWEST. U. S. Dept. Agr. Circ. 59, 20 p. 1929.

It has been repeatedly suggested that there is a relation between the nature of the skin and the keeping quality of an apple. The waxy covering of the skin should be of especial importance in a consideration of the matter of gaseous exchange between the interior of the fruit and the external atmosphere. At the present time storage scald is believed to be caused by gaseous or volatile products that are formed within the fruit as a result of abnormal respiratory processes.[4] Abnormal respiratory conditions may be produced by retarding the escape of carbon dioxide from the fruit and by hindering the entrance of oxygen. These changes might be associated to some extent with differences in permeability of the waxy coating on the surface of the fruit. It is not unreasonable, therefore, to assume that a possible correlation may exist between the physical nature of the apple coating and the development of storage scald.

In view of the fact that previous chemical work now enables us to determine quantitatively the surface constituents of the apple, and because of the importance of a further knowledge of these constituents to the solution of the above-mentioned problems, it seemed desirable to ascertain varietal differences and the effect of the stage of development of the fruit upon the percentage composition of the waxy coating.

PREVIOUS CHEMICAL INVESTIGATION OF APPLE-SURFACE COMPOUNDS

From the work mentioned above [5] it is evident that the surface coating may be separated into two distinct fractions, one soluble and the other insoluble in low-boiling petroleum ether. The soluble or low-melting portion is, at ordinary temperatures, a greenish lardaceous mass, becoming semiliquid but of a greasy consistency when rubbed between the fingers. The bulk of this petroleum-ether soluble portion has been shown to consist principally of the hydrocarbon triacontane ($C_{30}H_{62}$), melting at 63.5° to 64° C., and a much smaller quantity of the alcohol heptacosanol ($C_{27}H_{56}O$), melting at 81° to 81.5°. Small quantities of unidentified substances of a true oily nature probably are also present. The second fraction, the petroleum-ether insoluble portion, was found to consist of a greenish-yellow powder, resinous to the touch and very water repellent. In the pure crystalline state the compound melted at 285°. It was originally designated "malol," but subsequent work by Van der Haar [6] has shown it to be identical with ursolic acid (urson), previously isolated from the leaves of bearberry (*Arctostaphylos uva-ursi*). Further work by one of the writers [7] has confirmed this identification of malol with ursolic acid and the probable correctness of the empirical formula $C_{30}H_{48}O_3$ previously assigned to it.[8]

EXPERIMENTAL MATERIAL

Material for the analytical work herein reported was obtained from three sources: In 1926, from the variety orchard of the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.; in 1927, from the Arlington Experiment Farm and from Wenatchee, Wash.,

[4] BROOKS, C., COOLEY, J. S., and FISHER, D. F. APPLE-SCALD. Jour. Agr. Research 16: 195-217. 1919.
[5] SANDO, C. E. Op. cit. (See footnote 2.)
[6] VAN DER HAAR, A. W. UNTERSUCHUNGEN ÜBER DIE SAPONINE UND VERWANDTE KÖRPER. XIII. DIE IDENTITÄT VON MALOL MIT URSON (URSOLSÄURE). Rec. Trav. Chim. Pays-Bas 43: 548-549. 1924.
[7] SANDO, C. E. URSOLIC ACID. Jour. Biol. Chem. 90: 477-495. 1931.
[8] SANDO, C. E. Op. cit. (See footnote 2.)

except the McIntosh variety, which came from British Columbia. Apples obtained from the Arlington Experiment Farm were collected at three different stages of growth, designated, respectively, as early, medium, and mature. Material representing the mature stage was also placed in storage at 32° F. and is designated as "storage." The fruit from the Pacific Northwest, representing the mature stage, was picked at the usual harvesting time.[9]

METHODS OF ANALYSIS

PREPARATION OF SAMPLES

In order to obtain representative samples at each stage of growth and to avoid the necessity of analyzing a large number of individual fruits to determine existing variations, composite samples were used. Most of these composite samples represented 75 apples, although in certain cases, as indicated in the tables, fewer apples were used.

The method of collecting the fruit was fairly uniform. In most cases one tree supplied sufficient material throughout the entire season for each variety studied. Whenever two trees of the same variety were employed, adjacent ones were selected and approximately equal quantities were removed from each tree. Care was taken to obtain fruit from the outer branches having a southern or southeastern exposure. The apples, regardless of differences in size, were measured with calipers to the nearest millimeter for the purpose of recording individual diameters.

Samples were obtained from each apple by taking, with the aid of a cork borer, one disk each from the sunny and the shady side. In the early stage of some varieties, and even in the mature stage of such fruit as Grimes Golden and Rhode Island Greening, it was difficult to distinguish any marked difference in color between the two sides. In such instances a mixed sample was used. By using the same cork borer throughout the investigation, the disks obtained had a uniform diameter of 21.5 mm. As removed from the apple, the disk consisted of the waxlike coating, the ether-insoluble cuticle, epidermal cells, and adjacent tissues. (Fig. 1, A and B.) After immersing the disk in dilute hydrochloric acid (15 ml. concentrated acid to 2,000 ml. water) from 24 to 48 hours, the thin outer layer was separated from the flesh by means of tweezers. This layer consisted of the cuticle and its waxy coating together with the epidermal cells or a portion of them, to which the subepidermal cells sometimes adhered. (Fig. 1, C.) It was noticed that the cuticle was somewhat more difficult to remove from the sunny side than from the shady side of the same apple, and also that it could not be removed as easily from mature as from immature fruit. The cuticle was so difficult to remove in the described manner from mature fruit in certain cases, especially that of the York Imperial variety, that it was necessary to free it from the adhering tissues by careful scraping with the thumb-nail.

After separation, the cuticle was rinsed with tap water and then allowed to stand overnight in distilled water to insure complete removal of the hydrochloric acid. Each composite sample was then washed and sucked dry on a Büchner funnel and dried further over sulphuric acid in a vacuum desiccator. Samples thus prepared were stored in tightly stoppered bottles.

[9] The writers are indebted to H. C. Diehl, of the Bureau of Plant Industry, stationed at Wenatchee, Wash., for procuring this material and forwarding it to Washington, D. C.

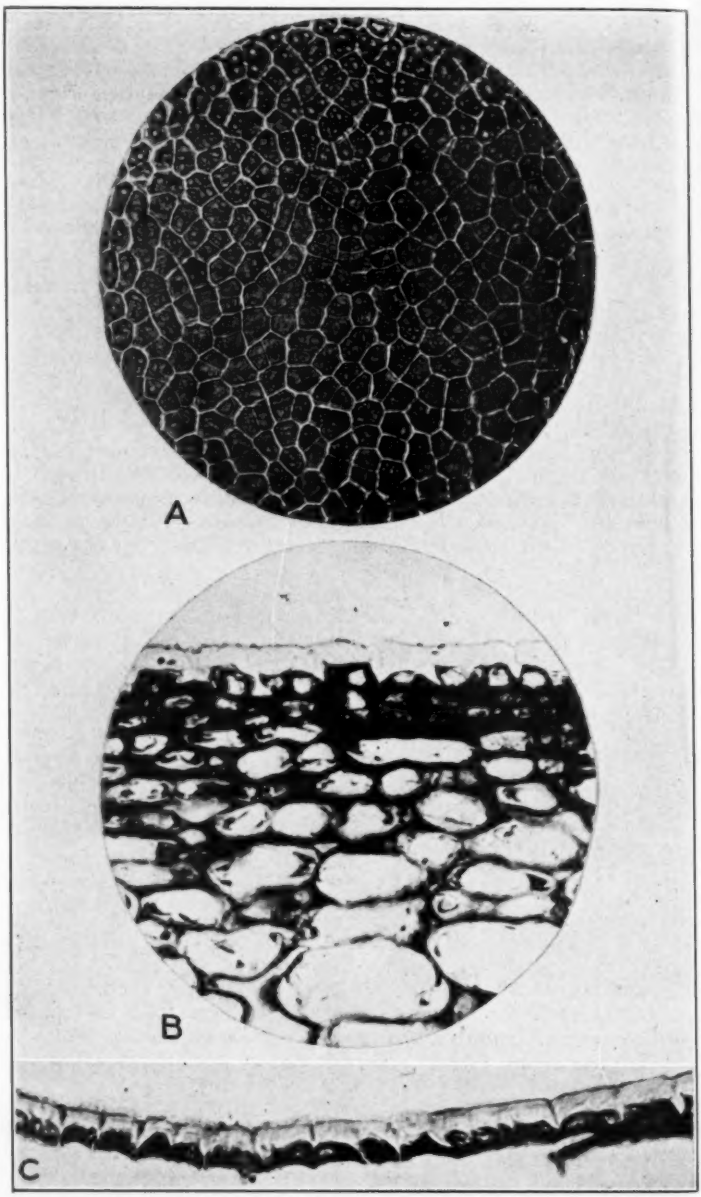

FIGURE 1.—Cuticular, epidermal, and subepidermal structure of Rome Beauty apple (New York). × 150: A, Surface view of cuticle, showing the underlying cellular structure of the epidermis; B, cuticle and section of the epidermis and adjacent tissues, showing thickness and extent of cuticle between epidermal cells; C, section of cuticle and all or a portion of the epidermal cells after removal with tweezers following treatment with dilute HCl. (These sections were kindly prepared by V. A. Pease, to whom the writers wish to express their thanks)

DETERMINATION OF TOTAL ETHER EXTRACT

The samples of waxy cuticle obtained in the manner described were placed in fritted Jena-glass thimbles and dried for two hours at 103° to 105° C. The samples in the thimbles were then placed in Soxhlets and extracted continuously for 45 hours with pure anhydrous ether. The flasks were changed and the samples again extracted for 15 hours with double-distilled 95 per cent alcohol. The residue obtained by evaporating the alcohol was dried and extracted three times with ether at the boiling temperature. The ether solution was filtered through ashless filter paper into the flask containing the original ether extract. The combined ether extracts were evaporated to dryness and the residues dried one and one-half hours to constant weight in an air oven at 105°. The combined dried residue constitutes the total ether extract. This was found to consist principally of ursolic acid, triacontane, and heptacosanol, together with small quantities of unidentified oily substances and plastid pigments.

DETERMINATION OF URSOLIC ACID

Inasmuch as ursolic acid is a hydroxy acid and forms a monosodium salt, it can be determined quantitatively by titration with alcoholic sodium hydroxide. The determination was carried out by adding approximately 50 ml. of 95 per cent alcohol and 1 ml. of 0.1 per cent phenolphthalein solution to the total ether extract and bringing the mixture to a boil so as to completely dissolve the residue. The hot solution was then titrated with approximately N/20 alcoholic sodium hydroxide, which was standardized at frequent intervals against benzoic acid. Blanks were run with each series of titrations and the correction applied to the final values.

DETERMINATION OF OILY FRACTION

The oily fraction, consisting mainly of triacontane and heptacosanol, undoubtedly contained small quantities of true oily substances and plastid pigments; but, on account of the very small quantity of total ether extract, the separation and quantitative determination of the individual constituents were obviously impossible. The weight of the oily fraction was obtained by subtracting the weight of ursolic acid from the weight of the total ether extract.

CALCULATION OF RESULTS

It was impracticable, for obvious reasons, to compute the analytical data on a basis of dry weight. The results are therefore reported on the basis of quantity of substance present on a unit area of apple surface, arbitrarily taken as 100,000 sq. mm. This area is equivalent to the area of 5 apples each 80 mm. in diameter, or to the area of 10 apples each 56.5 mm. in diameter. Since the apple surface is a curved one and the degree of curvature depends upon the size of the fruit, it was necessary to measure the equatorial diameter of each apple used in order to obtain the data necessary to calculate the total area of the experimental sample. For the calculation of the area of individual disks, it was necessary to apply the well-known geometrical formula for determining the area of the curved surface of a zone of one base. According to this formula, the area of the surface of a zone is equal to the product of its altitude (H) and the circumference of a great circle (πD), or

$$S = H.\pi D.$$

In the case of the apple disk the altitude could not be measured directly but was calculated from the radius (R) of the whole apple and from the radius (r) of the disk removed therefrom. The final formula, therefore, is

$$S = (R - \sqrt{R^2 - r^2}).\pi D.$$

PROGRESSIVE CHANGES IN SURFACE CONSTITUENTS DURING GROWTH AND STORAGE

The data showing progressive changes in surface constituents of the fruit during the period of growth and while in storage at 32° F. are assembled in Table 1. From this table it may be seen that the quantities of ursolic acid, oily fraction, and total either extract present at maturity and at the end of the storage period are invariably greater than those found in the early stage of growth for all varieties studied, for both the years 1926 and 1927. Data showing changes during storage are not available in all cases, but wherever given they indicate, with one exception, that the constituents increase from the time of picking to the end of the storage period. The progressive changes in constituents during growth and storage are brought out clearly in Figures 2 and 3.

While the quantities of both ursolic acid and the oily fraction become larger with increasing maturity, the oily fraction increases at a faster rate than does the ursolic acid; hence with advancing maturity there is in general a progressive increase in the percentage of the oily fraction in the total ether extract. (Table 1, columns 12 and 13.)

It is evident that most of the ursolic acid is deposited early in the growth of the apple, whereas the oily fraction, which is approximately 20 per cent lower than the ursolic acid in the early stage, is deposited at a uniformly higher rate throughout the later stages. This results in a gradual shift toward higher values of oily fractions (Table 1, columns 12 and 13) and lower values of ursolic acid in the total ether extract as the apple matures. A corresponding shift in the physical properties of the natural waxy coating must also accompany the shift in the ratio of the oily fraction to ursolic acid.

The change in physical properties which the waxy coating of apples undergoes as the fruit ripens may be a factor of some importance in the formation of certain odorous constituents. As the ratio of oily fraction to ursolic acid shifts, it is undoubtedly accompanied by a corresponding change in the physical properties of the natural waxy coating. Since the ursolic acid is dry, powdery, and porous and the oily fraction is pasty and greasy, it is natural to assume that the coating would become less permeable to gases as the percentage of oily fraction increases with advancing maturity.

A decrease in permeability might restrict the exchange of carbon dioxide and oxygen to such an extent that an intercellular-gas condition would be reached similar to that found by Thomas,[10] in which certain concentrations of these gases resulted in the formation of abnormal quantities of acetaldehyde.

[10] THOMAS, M. THE CONTROLLING INFLUENCE OF CARBON DIOXIDE. V. Biochem. Jour. 19: 927–947. 1925.

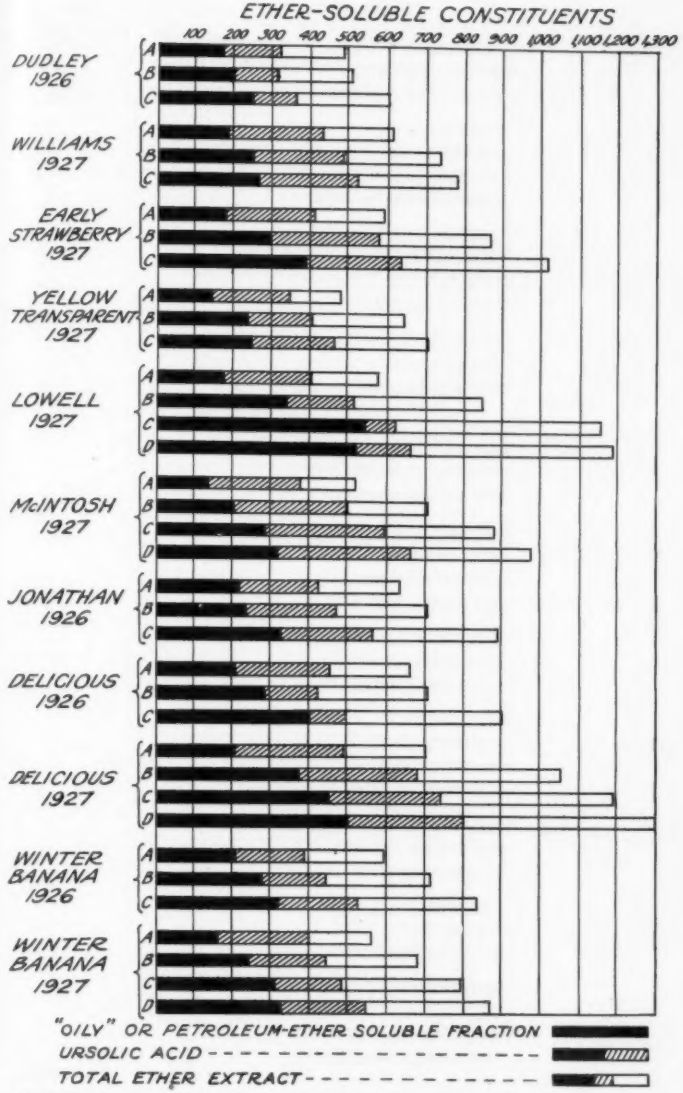

FIGURE 2.—Progressive changes in constituents during growth and storage expressed as milligrams per 100,000 sq. mm. of apple surface. A, B, C, and D refer to the early, medium, mature, and storage stages, respectively, shady side

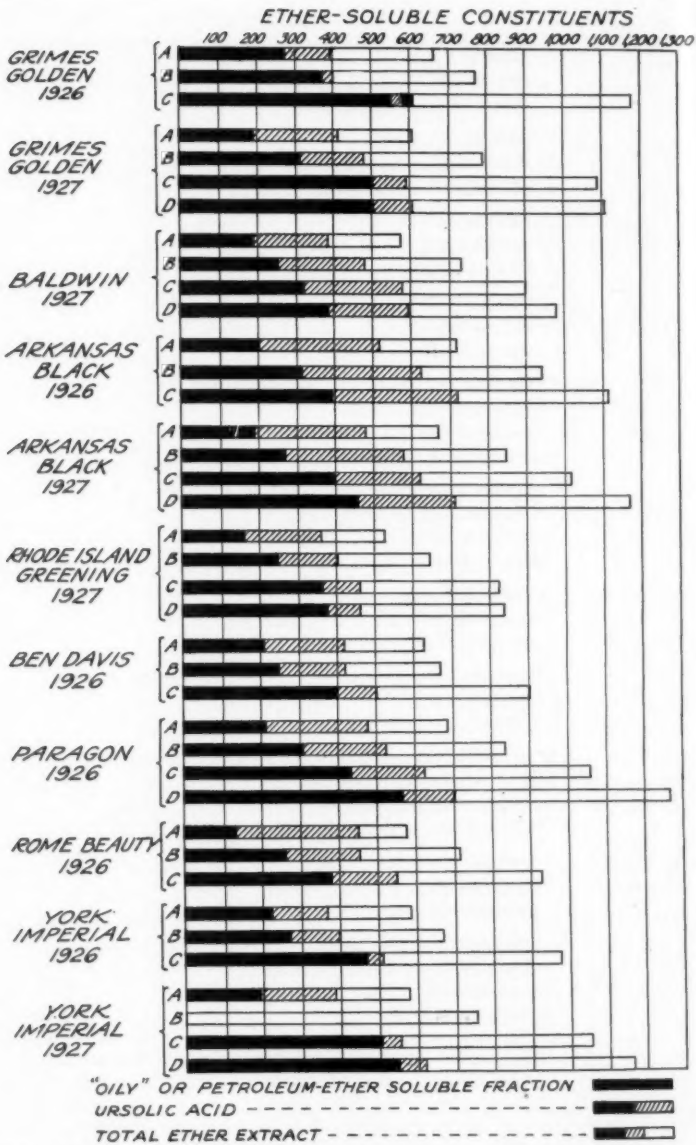

FIGURE 3.—Progressive changes in constituents during growth and storage expressed as milligrams per 100,000 sq. mm. of apple surface. A, B, C, and D refer to the early, medium, mature, and storage stages, respectively, shady side

TABLE 1.—*Progressive changes in the ether-soluble surface constituents of apple varieties during maturation and in storage*

Variety, year, and stage of growth	Date of sampling	Age from blooming	Minimum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 square millimeters of surface area						Oily or petroleum-ether soluble fraction in total ether extract	
					Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract			
					Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Summer varieties: [a]												
Dudley (1926)—		*Days*	*Mm.*	*Sq. mm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*
Early	6/15/26	47	43–50	[b] 19,213	318.5	274.3	167.6	183.7	486.1	458.0	34.5	40.1
Medium	7/27/26	89	63–77	27,924	310.5	273.9	198.7	196.2	509.2	470.1	39.0	41.7
Mature	8/11/26	104	62–81	27,867	359.9	366.0	244.3	257.6	604.2	623.6	40.4	41.3
Williams (1927)—												
Early	6/9/27	50	35–30	30,092	429.7	365.5	181.7	152.9	611.4	518.4	29.7	29.5
Mature	7/19/27	90	56–70	28,093	489.8	460.3	248.5	251.7	738.3	712.0	33.6	35.4
Storage	9/7/27	140	56–67	28,104	516.6	468.9	262.9	255.8	779.5	724.7	33.7	35.3
Early Strawberry (1927)—												
Early	6/6/27	47	31–35	31,135	407.9	335.0	178.6	152.6	586.5	487.6	30.5	31.3
Mature	7/18/27	89	47–56	28,526	577.1	432.3	291.7	249.3	868.8	681.6	33.6	36.6
Storage	9/14/27	147	47–56	28,598	630.8	467.5	386.8	341.0	1,017.6	808.5	38.0	42.2
Yellow Transparent (1927)—												
Early	6/6/27	47	30–30	30,733	345.7	330.4	140.6	139.0	486.3	469.4	28.9	29.6
Mature	7/11/27	82	54–67	28,185	[c] 408.0	[c] 408.0	[c] 234.9	[c] 234.9	[c] 642.9	[c] 642.9	[c] 36.5	[c] 36.5
Storage	9/13/27	146	51–65	28,234	[c] 459.7	[c] 459.7	[c] 243.7	[c] 243.7	[c] 703.4	[c] 703.4	[c] 34.6	[c] 34.6
Lowell (1927)—												
Early	6/6/27	47	30–36	31,044	404.2	361.4	171.1	194.5	575.3	555.9	29.8	35.0
Medium	7/21/27	92	55–65	28,175	[c] 508.9	[c] 508.9	[c] 338.6	[c] 338.6	[c] 847.5	[c] 847.5	[c] 39.9	[c] 39.9
Mature	8/23/27	125	62–90	27,881	[c] 619.8	[c] 619.8	[c] 538.4	[c] 538.4	[c] 1,158.2	[c] 1,158.2	[c] 46.5	[c] 46.5
Storage	10/18/27	181	64–83	27,948	[c] 660.9	[c] 660.9	[c] 517.7	[c] 517.7	[c] 1,178.6	[c] 1,178.6	[c] 43.9	[c] 43.9
Fall varieties: [a]												
McIntosh (1927)—												
Early	6/9/27	50	33–41	29,999	383.3	375.0	133.0	126.9	516.3	501.9	25.8	25.3
Medium	7/21/27	92	51–64	28,262	504.5	480.8	202.4	200.3	706.9	681.1	28.6	29.4
Mature	9/7/27	140	60–78	27,950	596.8	548.9	284.1	297.3	880.9	846.2	32.3	35.1
Storage	1/16/28	271	63–75	27,923	660.0	627.0	315.8	332.7	975.8	959.7	32.4	34.7
Jonathan (1926)—												
Early	6/24/26	56	33–43	30,037	420.1	408.8	215.0	172.8	635.2	581.6	33.8	29.7
Medium	7/29/26	91	46–57	28,595	471.0	420.3	235.7	251.4	706.7	671.7	33.4	37.4
Mature	9/25/26	149	60–76	27,958	567.3	500.1	324.1	355.6	891.4	855.6	36.4	41.6

[a] Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.
[b] Sample from 50 instead of 75 disks.
[c] Mixed sample; no difference discernible between shady and sunny sides of same fruit.

TABLE 1.—*Progressive changes in the ether-soluble surface constituents of apple varieties during maturation and in storage*—Continued

Variety, year, and stage of growth	Date of sampling	Age from blooming	Minimum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 square millimeters of surface area						Oily or petroleum-ether soluble fraction in total ether extract	
					Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract			
					Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Fall varieties—Continued.												
Delicious—												
1926:		*Days*	*Mm.*	*Sq. mm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*
Early	6/15/26	47	29–38	[b] 20,626	454.7		207.5		662.2	624.4	31.3	
Medium	7/27/26	89	45–55	28,782	420.7	423.5	288.7	293.9	709.4	717.4	40.7	41.0
Mature	10/6/26	160	63–73	27,983	505.7	508.9	398.8	410.6	904.6	919.6	44.1	44.6
1927:												
Early	6/13/27	54	31–39	30,628	496.3	452.5	208.6	210.6	704.9	663.1	29.6	31.8
Medium	7/26/27	97	47–61	28,423	682.1	614.6	370.5	358.8	1,052.6	973.4	35.2	36.9
Mature	9/21/27	154	59–73	27,997	747.6	732.3	448.7	496.1	1,196.3	1,228.4	37.5	40.4
Storage	1/24/28	279	60–72	28,004	800.3	786.3	500.3	545.7	1,300.6	1,332.0	38.5	41.0
Winter Banana—												
1926:												
Early	6/16/26	48	34–42	[b] 20,072	386.6	334.2	207.7	184.6	594.4	518.8	34.9	35.6
Medium	8/3/26	96	54–63	28,257	443.8	401.3	274.3	277.5	718.1	678.8	38.2	40.9
Mature	9/8/26	132	61–78	27,934	525.2	509.8	312.9	315.4	838.1	825.2	37.3	38.2
1927:												
Early	6/13/27	54	32–40	30,293	399.1	344.0	161.4	151.8	560.5	495.8	28.8	30.6
Medium	7/20/27	91	47–60	28,462	442.3	348.1	239.2	214.7	681.5	562.8	35.1	38.1
Mature	9/8/27	141	56–79	27,953	487.5	406.0	308.0	289.4	795.5	695.4	38.7	41.6
Storage	1/24/28	279	61–77	27,949	546.0	455.8	324.5	358.2	870.5	814.0	37.3	44.0
Grimes Golden—												
1926:												
Early	6/28/26	60	33–40	30,295	391.2	365.4	275.6	264.3	666.8	629.7	41.3	42.0
Medium	7/27/26	89	42–49	29,021	402.5	388.0	370.1	351.1	772.6	739.1	47.9	47.5
Mature	10/13/26	167	58–74	27,995	572.6	606.2	610.1	644.7	1,182.7	1,250.9	51.6	51.5
1927:												
Early	6/16/27	57	29–39	30,769	[c] 413.4	[c] 413.4	[c] 191.4	[c] 191.4	[c] 604.8	[c] 604.8	[c] 31.6	[c] 31.6
Medium	7/26/27	97	47–57	28,469	[c] 478.7	[c] 478.7	[c] 314.3	[c] 314.3	[c] 793.0	[c] 793.0	[c] 39.6	[c] 39.6
Mature	9/19/27	152	55–71	28,051	[c] 588.9	[c] 588.9	[c] 500.6	[c] 500.6	[c] 1,089.5	[c] 1,089.5	[c] 45.9	[c] 45.9
Storage	1/3/28	258	60–72	27,998	[c] 608.3	[c] 608.3	[c] 501.9	[c] 501.9	[c] 1,110.2	[c] 1,110.2	[c] 45.2	[c] 45.2
Late varieties:												
Baldwin (1927)—												
Early	6/9/27	50	33–43	29,991	386.4	333.4	190.4	164.0	576.8	497.4	33.0	33.0
Medium	8/1/27	103	55–69	28,135	479.4	382.0	255.2	254.5	734.6	636.5	34.7	40.0
Mature	9/20/27	153	65–83	27,836	579.4	459.8	322.6	361.7	902.0	821.5	35.8	44.0
Storage	1/14/28	269	67–84	27,825	594.8	495.2	387.1	376.3	981.9	871.5	39.4	43.2

Arkansas Black—												
1926:												
Early	6/15/26	47	30–37	[b] 20,662	515.5	491.3	204.2	201.8	719.7	693.1	28.4	29.1
Medium	8/7/26	100	49–60	28,442	626.6	569.6	316.4	317.8	943.0	887.4	33.6	35.8
Mature	9/25/26	149	61–73	27,980	722.3	634.0	395.6	482.1	1,117.9	1,116.2	35.4	43.2
1927:												
Early	6/13/27	54	30–36	31,079	480.1	438.0	189.2	192.1	669.3	630.1	28.3	30.5
Medium	7/25/27	96	47–57	28,928	578.0	510.9	270.0	305.3	848.0	816.2	31.8	37.4
Mature	9/26/27	159	60–75	27,970	624.5	600.6	397.2	396.1	1,021.7	996.7	38.9	39.7
Storage	2/8/28	294	60–72	28,007	713.6	674.7	459.5	515.5	1,173.1	1,190.2	39.2	43.3
Rhode Island Greening (1927)—												
Early	6/9/27	50	36–44	29,653	364.1	344.6	163.9	167.9	528.0	512.5	31.0	32.8
Medium	7/25/27	96	55–67	28,169	[c] 401.9	[c] 401.9	[c] 247.0	[c] 247.0	[c] 648.9	[c] 648.9	[c] 38.1	[c] 38.1
Mature	9/19/27	152	63–84	27,835	[c] 463.0	[c] 463.0	[c] 366.5	[c] 336.5	[c] 829.6	[c] 829.6	[c] 44.2	[c] 44.2
Storage	2/8/28	294	60–79	27,870	[c] 463.9	[c] 463.9	[c] 376.7	[c] 376.7	[c] 840.6	[c] 840.6	[c] 44.8	[c] 44.8
Ben Davis (1926)—												
Early	6/24/26	56	33–42	30,238	420.0	366.4	209.0	243.4	629.0	609.8	33.2	39.9
Medium	8/7/26	100	46–58	28,502	421.7	418.9	249.7	261.3	671.4	680.2	37.2	38.4
Mature	10/26/26	180	60–75	27,984	502.7	----------	400.2	----------	902.9	----------	44.3	----------
Paragon (1926)—												
Early	6/15/26	47	32–41	[b] 20,144	478.5	445.0	211.5	179.4	690.0	624.4	30.6	28.7
Medium	8/3/26	96	50–60	28,392	528.7	522.7	310.6	300.4	839.3	823.1	37.0	36.5
Mature	10/28/26	182	63–79	27,877	627.0	----------	433.7	----------	1,060.7	----------	40.9	----------
Storage	2/21/27	298	64–77	27,931	702.0	659.4	570.3	547.7	1,272.3	1,207.1	44.8	45.4
Rome Beauty (1926)—												
Early	6/24/26	52	34–42	29,827	454.7	394.6	127.1	168.0	581.7	562.6	21.8	29.9
Medium	8/4/26	93	48–60	28,479	456.8	434.0	264.0	185.7	720.8	619.7	36.6	30.0
Mature	11/2/26	183	66–81	27,842	552.8	518.0	382.2	407.7	934.6	925.6	40.9	44.0
York Imperial—												
1926:												
Early	6/28/26	60	37–50	29,353	369.0	380.6	223.5	217.4	592.5	596.6	37.7	36.2
Medium	7/29/26	91	48–60	28,535	400.5	406.5	273.7	312.5	674.2	719.0	40.6	43.5
Mature	11/2/26	187	67–83	27,870	514.2	495.5	470.0	406.5	984.2	902.0	47.8	45.1
1927:												
Early	6/16/27	57	31–35	31,148	389.7	362.7	193.5	184.3	583.2	547.0	33.2	33.7
Medium	8/15/27	117	50–59	28,411	----------	415.4	----------	283.7	759.3	699.1	----------	40.6
Mature	10/17/27	180	60–76	28,042	558.4	420.8	506.4	508.5	1,064.8	929.3	47.6	54.7
Storage	3/3/28	318	61–70	28,050	623.9	504.4	550.8	560.4	1,174.7	1,064.8	46.9	52.6
Varieties from Pacific Northwest:												
McIntosh [d] (1927)—												
Mature	10/7/27	----------	71–83	27,804	807.6	729.6	420.3	441.8	1,227.9	1,171.4	34.2	37.7
Jonathan [e] (1927)—												
Mature	10/7/27	----------	64–76	27,908	542.8	530.9	299.6	298.9	842.4	829.8	35.6	36.0
Delicious [e] (1927)—												
Mature	10/6/27	----------	68–80	27,849	437.5	399.7	269.1	271.7	706.6	671.4	38.1	40.5
Grimes Golden [e] (1927)—												
Mature	10/6/27	----------	62–71	27,974	658.2	610.1	421.0	444.0	1,079.2	1,054.1	39.0	42.1
Winter Banana [e] (1927)—												
Mature	9/27/27	----------	71–82	27,796	619.1	632.7	443.5	469.0	1,062.6	1,101.7	41.7	42.6

[b] Sample from 50 instead of 75 disks.
[c] Mixed sample; no difference discernible between shady and sunny sides of some fruit.
[d] From British Columbia.
[e] From Wenatchee, Wash.

TABLE 1.—*Progressive changes in the ether-soluble surface constituents of apple varieties during maturation and in storage*—Continued

Variety, year, and stage of growth	Date of sampling	Age from blooming	Minimum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 square millimeters of surface area						Oily or petroleum-ether soluble fraction in total ether extract	
					Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract			
					Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Summer varieties: [a]												
Arkansas Black [c] (1927)—		*Days*	*Mm.*	*Sq. mm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*
Mature	10/27/27		69–80	27,816	542.5	533.5	392.3	364.4	934.8	897.9	42.0	40.6
Esopus Spitzenburg [c] (1927)—												
Mature	10/28/27		58–75	27,931	474.0	474.0	451.5	451.5	925.5	925.5	48.8	48.8
Mean summer (1927):												
Early		48			396.9	348.1	168.0	159.8	564.9	507.8	29.7	31.4
Mature		96			523.7	480.1	328.4	318.6	852.0	798.7	37.6	38.8
Storage		154			567.0	514.2	352.8	339.6	919.8	853.8	37.6	39.0
Mean fall (1926):												
Early		53			413.2	369.5	226.4	207.2	639.6	588.6	35.3	35.8
Medium		91			434.5	408.3	292.2	293.5	726.7	701.8	40.0	41.7
Mature		152			542.7	531.2	411.5	431.6	954.2	962.8	42.4	44.0
Mean fall (1927):												
Early		54			423.0	396.2	173.6	170.2	596.6	566.4	29.0	29.8
Medium		94			526.9	480.6	281.6	272.0	808.5	752.6	34.6	36.0
Mature		147			605.2	569.0	385.4	395.9	990.6	964.9	38.6	40.8
Storage		272			653.6	619.4	410.6	434.6	1,064.3	1,054.0	38.4	41.2
Mean late (1926):												
Early		52			447.5	415.6	195.1	202.0	642.6	617.3	30.3	32.8
Medium		96			486.9	470.3	282.9	275.5	769.7	745.9	37.0	36.8
Mature		176			583.8	549.2	416.3	432.1	1,000.1	981.3	41.9	44.1
Mean late (1927):												
Early		53			405.1	369.7	184.2	177.1	589.3	546.8	31.4	32.5
Medium		103			486.4	427.6	257.4	272.6	747.7	700.2	34.9	39.0
Mature		161			556.3	486.0	398.2	408.2	954.5	894.3	41.6	45.6
Storage		294			599.0	534.0	443.5	457.2	1,042.6	991.8	42.6	46.0
Mean Pacific Northwest (1927):												
Mature					583.1	558.6	385.3	391.6	968.4	950.2	39.9	41.2

[a] Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.

[c] From Wenatchee, Wash.

DIFFERENCES BETWEEN SHADY AND SUNNY SIDES OF SAME FRUIT

In general, the shady side of an apple as compared with the sunny side of the same apple possesses larger quantities of ursolic acid and total ether extract at all stages of growth and during the storage period. In view of the fact that the shady side contains a larger quantity of total ether extract, and since there seems to be no significant difference in oily fraction between the two sides, it follows that the percentage of oily fraction in the total ether extract should be lower on the shady side, as is actually the case.

The differences between the shady and sunny sides that have just been discussed are of particular interest in connection with the changes in quantity of constituents accompanying maturation. It has been pointed out that quantities of the various constituents increase with increasing maturity, but it may be noted that the actual values for ursolic acid and total ether extract are slightly smaller on the sunny side, which is generally considered the most mature portion of the apple. On the other hand, the percentage of oily fraction in the total ether extract is higher on the sunny side, thus conforming with the rule that the percentage of oily fraction increases with maturity.

It may be concluded, therefore, not only that the percentage of oily fraction is a criterion of maturity when apples of different ages are compared, but also that the higher percentages of this fraction on the sunny side of the fruit indicate a more mature condition of the sunny side as compared with the shady side of the same fruit.

DIFFERENCES BETWEEN FRUIT COLLECTED IN 1926 AND 1927

With respect to the mean values for the quantities of the various constituents representing the fall and late groups, it is apparent that no marked differences exist between the two years 1926 and 1927.

However, when the values for certain individual varieties are considered, it is found that some were higher in 1926, others higher in 1927, and still others practically the same for both years. For example, in comparing data for the shady side only, it is noted that Arkansas Black at picking maturity was higher in ursolic acid (722.3 : 624.5 mgm.) and in total ether extract (1,117.9 : 1,021.7 mgm.) for 1926 than for 1927, and practically identical in the oily fraction for the two years (395.6 : 397.2 mgm.). York Imperial, on the other hand, was lower in ursolic acid (514.2 : 558.4 mgm.) and in the oily fraction (470.0 : 506.4 mgm.), and consequently in the total ether extract (984.2 : 1,064.8 mgm.), for 1926 than for 1927. Delicious was markedly lower in ursolic acid (505.7 : 747.6 mgm.), somewhat lower in oily fraction (398.8 : 448.7 mgm.), and considerably lower in total ether extract (904.6 : 1,196.3 mgm.) for 1926 than for 1927. Winter Banana shows higher ursolic acid (525.2 : 487.5 mgm.) and higher total ether extract (838.1 : 795.5 mgm.) in 1926 than in 1927, and about the same oily fraction (312.9 : 308.0 mgm.) for the two years. Grimes Golden shows practically the same quantity of ursolic acid (572.6 : 588.9 mgm.) for the two years, but a much higher value in oily fraction (610.1 : 500.6 mgm.) for 1926, which in turn is responsible for the higher value for the total ether extract (1,182.7 : 1,089.5 mgm.) in 1926 as compared with that in 1927.

DIFFERENCES AMONG VARIETIES AT PICKING MATURITY

It is obvious from a consideration of the data presented in Table 1 that varietal differences are found with respect to quantities of constituents present at the different stages of maturity. Limiting the present discussion to the mature stage, and averaging the data for the shady and sunny sides of 1926 and 1927 fruit, the following varietal arrangement with respect to quantities in milligrams per unit area of ursolic acid is obtained:

Dudley, 363; Yellow Transparent, 408; Rhode Island Greening, 463; Williams, 475; Winter Banana, 482.1; York Imperial, 497.2; Ben Davis, 502.7; Early Strawberry, 504.7; Baldwin, 519.6; Jonathan, 533.7; Rome Beauty, 535.4; McIntosh, 572.8; Grimes Golden, 589.2; Lowell, 619.8; Delicious, 623.6; Paragon, 627; and Arkansas Black, 645.4.

When the quantities of oily fraction are similarly averaged, the arrangement of the varieties is as follows: Yellow Transparent, 234.9; Williams, 250.1; Dudley, 251; Early Strawberry, 270.5; McIntosh, 290.7; Winter Banana, 306.4; Jonathan, 339.8; Baldwin, 342.2; Rhode Island Greening, 366.5; Rome Beauty, 395; Ben Davis, 400.2; Arkansas Black, 417.8; Paragon, 433.7; Delicious, 438.6; York Imperial, 472.8; Lowell, 538.4; and Grimes Golden, 564.

With respect to the total ether extract, the arrangement is as follows: Dudley, 613.9; Yellow Transparent, 642.9; Williams, 725.2; Early Strawberry, 775.2; Winter Banana, 788.6; Rhode Island Greening, 829.6; Baldwin, 861.8; McIntosh, 863.6, Jonathan, 873.5; Ben Davis, 902.9; Rome Beauty, 930.1; York Imperial, 970.1; Paragon, 1,060.7; Delicious, 1,062.2; Arkansas Black, 1,063.1; Grimes Golden, 1,153.2; and Lowell, 1,158.2.

QUANTITIES OF CONSTITUENTS EXPRESSED AS PERCENTAGES OF THE TOTAL CONSTITUENTS FOUND AT THE MATURE STAGE AND AFTER STORAGE

The data showing the quantities of the different fractions at various stages of growth, expressed as percentages of the total corresponding constituents at maturity and after storage are presented in Tables 2 and 3. From these tables it may be seen that a very large proportion of the several constituents is formed at an early stage in the development of the apple.

Considering the quantity of ursolic acid present at the mature stage as 100 per cent, it is interesting to note that approximately 75 per cent is deposited by the time the early stage is reached, or about 50 days from blooming, at which time the fruit has attained a size approximately 30 to 50 mm. in diameter. For the same period of growth the quantity of oily fraction laid down represents about 50 per cent of the total quantity present at the mature stage. From the data in Table 2 it is apparent that approximately 65 per cent of the total ether-soluble constituents are deposited within the first 50 days of growth.

TABLE 2.—*Surface constituents of apple varieties at different stages of growth expressed as percentages of corresponding constituents found at picking maturity*

Variety, year, and stage of growth	Age from blooming	Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract	
		Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Summer varieties:[a]							
Dudley (1926)—	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Early	47	88.5	74.9	68.6	71.3	80.4	73.4
Medium	89	86.3	74.8	81.3	76.2	84.3	75.4
Mature	104	100.0	100.0	100.0	100.0	100.0	100.0
Williams (1927)—							
Early	50	87.7	79.4	73.1	60.7	82.8	72.8
Mature	90	100.0	100.0	100.0	100.0	100.0	100.0
Early Strawberry (1927)—							
Early	47	70.7	77.5	61.2	61.2	67.5	71.5
Mature	89	100.0	100.0	100.0	100.0	100.0	100.0
Yellow Transparent (1927)—							
Early	47	84.7	81.0	59.8	59.2	75.6	73.0
Mature	82	100.0	100.0	100.0	100.0	100.0	100.0
Lowell (1927)—							
Early	47	65.2	58.3	31.8	36.1	49.7	48.0
Medium	92	[b] 82.1	[b] 82.1	[b] 62.9	[b] 62.9	[b] 73.2	[b] 73.2
Mature	125	100.0	100.0	100.0	100.0	100.0	100.0
Mean (1927)—							
Early	48	77.1	74.0	56.5	54.3	68.6	66.3
Mature	96	100.0	100.0	100.0	100.0	100.0	100.0
Fall varieties:[a]							
McIntosh (1927)—							
Early	50	64.2	68.3	46.8	42.7	58.6	59.3
Medium	92	84.5	87.6	71.2	67.4	80.2	80.5
Mature	140	100.0	100.0	100.0	100.0	100.0	100.0
Jonathan (1926)—							
Early	56	74.0	81.7	66.3	48.6	71.2	68.0
Medium	91	83.0	84.0	72.7	70.7	79.3	78.5
Mature	149	100.0	100.0	100.0	100.0	100.0	100.0
Delicious—							
1926:							
Early	47	89.9	------	52.0	------	73.2	67.9
Medium	89	83.2	83.2	72.4	71.6	78.4	78.0
Mature	160	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	54	66.4	61.8	46.5	42.4	58.9	54.0
Medium	97	91.2	83.9	82.6	72.3	88.0	79.2
Mature	154	100.0	100.0	100.0	100.0	100.0	100.0
Winter Banana—							
1926:							
Early	48	73.6	65.6	66.4	58.5	70.9	62.9
Medium	96	84.5	78.7	87.7	88.0	85.7	82.2
Mature	132	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	54	81.9	84.7	52.4	50.9	70.4	71.3
Medium	91	90.7	85.7	77.7	72.0	85.7	80.9
Mature	141	100.0	100.0	100.0	100.0	100.0	100.0
Grimes Golden—							
1926:							
Early	60	68.3	60.3	45.2	41.0	56.4	50.3
Medium	89	70.3	64.0	60.7	54.4	65.3	59.1
Mature	167	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	57	[b] 70.2	[b] 70.2	[b] 38.2	[b] 38.2	[b] 55.5	[b] 55.5
Medium	97	[b] 81.3	[b] 81.3	[b] 62.8	[b] 62.8	[b] 72.8	[b] 72.8
Mature	152	100.0	100.0	100.0	100.0	100.0	100.0
Mean—							
1926:							
Early	53	76.4	69.2	57.5	49.4	67.9	62.3
Medium	91	80.2	77.5	73.4	71.2	77.2	74.4
Mature	152	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	54	70.7	71.2	46.0	43.6	60.8	60.0
Medium	94	86.9	84.6	73.6	68.6	81.7	78.4
Mature	147	100.0	100.0	100.0	100.0	100.0	100.0

[a] Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.
[b] Mixed sample; no difference discernible between shady and sunny sides of same fruit.

TABLE 2.—*Surface constituents of apple varieties at different stages of growth expressed as percentages of corresponding constituents found at picking maturity*—Continued

Variety, year, and stage of growth	Age from blooming	Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract	
		Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Late varieties:[a]							
Baldwin (1927)—	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Early	50	66.7	72.5	59.0	45.3	63.9	69.5
Medium	103	82.7	83.1	79.1	70.4	81.4	77.5
Mature	153	100.0	100.0	100.0	100.0	100.0	100.0
Arkansas Black—							
1926:							
Early	47	71.4	77.5	51.6	41.8	64.4	62.1
Medium	100	86.8	89.8	80.0	65.9	84.4	79.5
Mature	149	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	54	76.9	72.9	47.6	48.5	65.5	63.2
Medium	96	92.5	85.1	68.0	77.1	83.0	81.9
Mature	159	100.0	100.0	100.0	100.0	100.0	100.0
Rhode Island Greening (1927)—							
Early	50	78.6	74.4	44.7	45.8	63.7	61.8
Medium	96	[b] 86.8	[b] 86.8	[b] 67.4	[b] 67.4	[b] 78.2	[b] 78.2
Mature	152	100.0	100.0	100.0	100.0	100.0	100.0
Ben Davis (1926)—							
Early	56	83.5	.....	52.2	.....	69.7	.....
Medium	100	83.9	.....	62.4	.....	74.4	.....
Mature	180	100.0	100.0	100.0	100.0	100.0	100.0
Paragon (1926)—							
Early	47	76.3	.....	48.8	.....	65.0	.....
Medium	96	84.3	.....	71.6	.....	79.1	.....
Mature	182	100.0	100.0	100.0	100.0	100.0	100.0
Rome Beauty (1926)—							
Early	52	82.2	76.2	33.2	41.2	62.2	60.8
Medium	93	82.6	83.8	69.1	45.5	77.1	67.0
Mature	183	100.0	100.0	100.0	100.0	100.0	100.0
York Imperial—							
1926:							
Early	60	71.8	76.8	47.6	53.5	60.2	66.1
Medium	91	77.9	82.0	58.2	76.9	68.5	79.7
Mature	187	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	57	69.8	86.2	38.2	36.2	54.8	58.9
Medium	117	.....	98.7	.....	55.8	71.3	75.2
Mature	180	100.0	100.0	100.0	100.0	100.0	100.0
Mean—							
1926:							
Early	52	77.0	76.8	46.7	45.5	64.3	63.0
Medium	96	83.1	85.2	68.3	62.8	76.7	75.4
Mature	176	100.0	100.0	100.0	100.0	100.0	100.0
1927:							
Early	53	73.0	76.5	47.4	44.0	62.0	61.1
Medium	103	87.3	88.4	71.5	67.7	78.5	78.2
Mature	161	100.0	100.0	100.0	100.0	100.0	100.0

[a] Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.
[b] Mixed sample; no difference discernible between shady and sunny sides of same fruit.

If the quantity of constituents formed by the end of the storage period, instead of the quantity found at the mature stage, is taken to represent 100 per cent, it is seen (Table 3) that about 68 per cent of ursolic acid is deposited within the first 50 days of growth, while approximately 45 per cent of oily fraction is deposited within the same period. Inasmuch as no data are available relative to the quantities of constituents formed previous to the date of early-stage sampling, it would seem to be of further interest to study the composition, quantity, and rate of deposition at closer intervals after blooming.

TABLE 3.—*Surface constituents of apple varieties at different stages of growth expressed as percentages of corresponding constituents found at the end of the storage period*

Variety, year, and stage of growth	Age from blooming	Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract	
		Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Summer varieties: [a]							
Williams (1927)—	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Early	50	83.2	77.9	69.1	59.8	78.4	71.5
Mature	90	94.8	98.2	94.5	98.4	94.7	98.2
Storage	140	100.0	100.0	100.0	100.0	100.0	100.0
Early Strawberry (1927)—							
Early	47	64.7	71.6	46.2	44.8	57.6	60.3
Mature	89	91.5	92.5	75.4	73.1	85.4	84.3
Storage	147	100.0	100.0	100.0	100.0	100.0	100.0
Yellow Transparent (1927)—							
Early	47	75.2	71.9	57.7	57.0	69.1	66.7
Mature	82	[b] 88.8	[b] 88.8	[b] 96.4	[b] 96.4	[b] 91.4	[b] 91.4
Storage	146	100.0	100.0	100.0	100.0	100.0	100.0
Lowell (1927)—							
Early	47	61.2	54.7	33.0	37.6	48.8	47.2
Medium	92	[b] 77.0	[b] 77.0	[b] 65.4	[b] 65.4	[b] 71.9	[b] 71.9
Mature	125	[b] 93.8	[b] 93.8	-------	-------	[b] 98.3	[b] 98.3
Storage	181	100.0	100.0	100.0	100.0	100.0	100.0
Mean (1927)—							
Early	48	71.1	69.0	51.5	49.8	63.5	61.4
Mature	96	92.2	93.3	88.8	89.3	92.4	93.0
Storage	154	100.0	100.0	100.0	100.0	100.0	100.0
Fall varieties: [a]							
McIntosh (1927)—							
Early	50	58.1	59.8	42.1	38.1	52.3	52.3
Medium	92	76.4	76.7	64.1	60.2	72.4	71.0
Mature	140	90.4	87.5	90.0	89.4	90.3	88.2
Storage	271	100.0	100.0	100.0	100.0	100.0	100.0
Delicious (1927)—							
Early	54	62.0	57.5	41.7	38.6	54.2	49.8
Medium	97	85.2	78.2	74.0	65.8	80.9	73.1
Mature	154	93.4	93.1	89.7	90.9	92.0	92.2
Storage	279	100.0	100.0	100.0	100.0	100.0	100.0
Winter Banana (1927)—							
Early	54	73.1	75.5	49.7	42.4	64.4	60.9
Medium	91	81.0	75.4	73.7	59.9	78.3	69.1
Mature	141	89.3	89.1	94.9	83.3	91.4	85.4
Storage	279	100.0	100.0	100.0	100.0	100.0	100.0
Grimes Golden (1927)—							
Early	57	[b] 68.0	[b] 68.0	[b] 38.1	[b] 38.1	[b] 54.5	[b] 54.5
Medium	97	[b] 78.7	[b] 78.7	[b] 62.6	[b] 62.6	[b] 71.4	[b] 71.4
Mature	152	[b] 96.8	[b] 96.8	[b] 99.7	[b] 99.7	[b] 98.1	[b] 98.1
Storage	258	100.0	100.0	100.0	100.0	100.0	100.0
Mean (1927)—							
Early	54	65.3	65.2	42.9	39.3	56.5	54.4
Medium	94	80.3	77.5	68.6	62.1	75.8	71.2
Mature	147	92.5	91.6	93.6	90.8	93.0	91.0
Storage	272	100.0	100.0	100.0	100.0	100.0	100.0
Late varieties: [a]							
Baldwin (1927)—							
Early	50	65.0	67.3	49.2	43.6	58.7	57.1
Medium	103	80.6	77.1	65.9	67.6	74.8	73.0
Mature	153	97.4	92.8	83.3	96.1	91.9	94.3
Storage	289	100.0	100.0	100.0	100.0	100.0	100.0
Arkansas Black (1927)—							
Early	54	67.3	64.9	41.2	37.3	57.0	52.9
Medium	96	81.0	75.7	58.8	59.2	72.3	68.6
Mature	159	87.5	89.0	86.4	76.8	87.1	83.7
Storage	294	100.0	100.0	100.0	100.0	100.0	100.0
Rhode Island Greening (1927)—							
Early	50	78.5	74.3	43.5	44.6	62.8	61.0
Medium	96	[b] 86.6	[b] 86.6	[b] 65.6	[b] 65.6	[b] 77.2	[b] 77.2
Mature	152	[b] 99.8	[b] 99.8	[b] 97.3	[b] 97.3	[b] 98.7	[b] 98.7
Storage	294	100.0	100.0	100.0	100.0	100.0	100.0
Paragon (1926)—							
Early	47	68.2	67.5	37.1	32.8	54.2	51.7
Medium	96	75.3	79.3	54.5	54.8	66.0	68.2
Mature	182	89.3	-------	76.0	-------	83.4	-------
Storage	298	100.0	100.0	100.0	100.0	100.0	100.0

[a] Grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C.
[b] Mixed sample; no difference discernible between shady and sunny sides of same fruit.

TABLE 3.—*Surface constituents of apple varieties at different stages of growth expressed as percentages of corresponding constituents found at the end of the storage period*—Continued

Variety, year, and stage of growth	Age from blooming	Ursolic acid		Oily or petroleum-ether soluble fraction		Total ether extract	
		Shady side	Sunny side	Shady side	Sunny side	Shady side	Sunny side
Late varieties—Continued.							
York Imperial (1927)—	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Early	57	62.5	71.9	35.1	32.9	49.6	51.4
Medium	117	------	82.4	------	50.6	64.6	65.6
Mature	180	89.5	83.4	91.9	90.7	90.6	87.3
Storage	318	100.0	100.0	100.0	100.0	100.0	100.0
Mean (1927)—							
Early	53	68.3	69.6	42.2	39.6	57.0	55.6
Medium	103	82.4	80.4	63.4	60.8	72.2	71.1
Mature	161	93.2	91.2	89.7	90.2	91.8	91.0
Storage	294	100.0	100.0	100.0	100.0	100.0	100.0

SUMMARY AND CONCLUSIONS

With the particular object of determining the progressive changes that occur in the composition of the waxlike coating on the surface of the apple, analyses were made of summer, fall, and late varieties at several stages of growth and after storage at 32° F.

The results of these analyses, expressed in milligrams per 100,000 sq. mm. of apple surface, indicate that in general there is an increase in ursolic acid, oily fraction, and total ether extract throughout the growing period and during storage.

The oily fraction increases at a faster rate than ursolic acid, thus explaining the progressive increase in the percentage of oily fraction in the total ether extract with advancing maturity.

A change in the physical properties of the natural waxy coating undoubtedly accompanies the shift in the ratio of oily fraction to ursolic acid. Such a change probably not only affects the permeability of the coating to gases but also influences the effectiveness of spray applications and the ease of spray-residue removal.

In general, larger quantities of ursolic acid and total ether extract are deposited on the shady side than on the sunny side of the same apple, whereas there seems to be no appreciable difference in quantity of oily fraction. As a result, the percentage of oily fraction in the total ether extract is lower on the shady side.

Comparative data for varieties collected in the years 1926 and 1927 indicate that values for ursolic acid, oily fraction, and total ether extract may show variations from year to year, depending most probably on whether the season was favorable or adverse to the normal development of a particular variety.

It has been established that there are considerable variations among varieties with respect to quantities of ursolic acid, oily fraction, and total ether extract found at picking maturity.

Considering the average quantity of each substance deposited by the time the early stage is reached (approximately 40 to 60 days from blooming) as a percentage of the amount of the same substance present at maturity and at the end of the storage season, respectively, the following approximate values are found: For ursolic acid, 75 and 68 per cent; for oily fraction, 50 and 45 per cent; and for total ether extract, 65 and 58 per cent, respectively.

AN ANALYSIS OF THE EFFECTS OF DIPLODIA INFECTION AND TREATMENT OF SEED CORN[1]

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INTRODUCTION

The effects of various seed-borne seedling diseases caused by dry-rot organisms, and the effects of seed disinfection upon field stands and acre yields of corn have been rather extensively investigated by a number of State experiment stations and the United States Department of Agriculture. The literature has recently been so fully summarized by Koehler and Holbert (*6*)[2] that a review and enumeration of the many references would here seem unnecessary. It may be concluded from published reports that the prevalence and seriousness of these diseases vary regionally and seasonally with climate and cultural practice. In Nebraska they seem to offer no important crop-production problem with seed corn that is selected with the usual care.

When heavily infected viable seed has been selected and planted experimentally, however, the customary effects of curtailed stands have followed. Likewise, disinfection of such seed with commercial organic mercury compounds has resulted in improved field germination.

The most obvious result of planting viable infected seed is a stand shortage due to the death of many individuals caused by the rot organisms before emergence. The extent to which surviving infected plants might be depressed in their growth and productivity does not appear to have been clearly established. It has been the primary purpose of the investigation herein reported to determine this for the progeny from seed which was naturally infected with the most common of these seedling-blight organisms, *Diplodia zeae* (Schw.) Lév. The procedure has been that of statistical analysis.

Under field-planting conditions the diseased plants from such seed grow interspersed among healthy plants. It may be assumed that such a population will be less uniform in development than one with fewer diseased plants. As a concise expression of such effects, the means and their coefficients of variability have been calculated for various plant characters in the case of progenies from treated and untreated diseased and nearly disease-free seed. The characters considered were seedling weight, plant height at three stages of growth, days from planting to silking, shrinkage of ear corn, shelling percentage, and grain yield. In addition, the effects of the disease and of seed treatment on germination, seedling emergence, lodging, suckers, barren plants, 2-eared stalks, and smut in the resulting crop were determined. A record was also kept of the individual development of plants which gave initial evidence of being diseased as compared with adjacent healthy plants.

[1] Received for publication Jan. 6, 1931; issued June, 1931. Contribution from the Department of Agronomy, Nebraska Agricultural Experiment Station, as paper No. 100 of the Journal Series.

[2] Reference is made by number (italic) to Literature Cited, p. 749.

Supplemental to the investigations enumerated above concerning the comparative behavior of the progenies from diseased and disease-free seed, a study was made of the effects of seed treatment on farm-selected or "planter-box" seed corn.

EXPERIMENTAL METHODS OF THE STATISTICAL STUDY

The seed used in this investigation was supplied by J. R. Holbert, of the United States Department of Agriculture. It was a double cross of uniform hybrid origin known as Hybrid No. 365. Two lots of seed differing in respect to infection with *Diplodia zeae* had been selected by means of the individual ear germinator test as described by Holbert et al. (*2*). Although one contained approximately 90 per cent infected kernels and the other less than 0.5 per cent, both had the appearance of good seed. Hereafter these will be referred to as diseased and healthy seed. Half of each lot was thoroughly treated with the mercuric disinfectant known as Semesan Jr. at the rate of 2 ounces per bushel. Thus the four lots of seed concerned consisted of untreated and treated diseased seed and untreated and treated healthy seed.

A laboratory germination test of the diseased and healthy seed was made by the rag-doll method. Field germination was determined for all four lots of seed by counting the number of seedlings which had emerged above the surface of the ground at intervals of 9, 12, 15, and 18 days after planting. Because the exact number of seeds planted was known, the counts of emerged seedlings were readily converted into percentage of field germination.

The tests were conducted in 1929 on the experiment station farm. Planting was done by hand on May 16 in surface-planted plots containing either four or five rows spaced 3.5 feet apart. The seeds were dropped every 14 inches in the row. All rows were 39.7 feet long and, when no skips occurred, contained 34 plants. The results were based on the interior rows of the plots only, and two plants at either end of each row were discarded. Ten systematic replications were provided for all plantings, in order that variability due to soil heterogeneity would be equally distributed. A full stand would thus provide populations of either 600 or 900 plants from each of the plantings for statistical study according to whether the plots contained four or five rows.

Based on the difference in laboratory germination and on previous experiences with similar seed, it seemed probable that unequal stands would result from the various lots of seed, if planted at a single uniform rate. Data taken from such stands would measure variability caused not only by Diplodia or seed treatment, but also by a variable competition within plots of differing population densities.

It seemed desirable so to adapt the plantings that the data obtained would show specifically the variability caused by (1) Diplodia or treatment alone and (2) the combined effects of Diplodia or seed treatment and the associated stand differences. Consequently plantings were made at two rates, a normal rate and a double rate. The plots planted at the double rate were later thinned; those planted at the normal rate were not. To make the desired comparisons, a total of 10 replicate series of 8 plots each was required. Plots 1 and 2 were respectively planted to untreated and treated diseased seed at the standard rate of one seed every 14 inches in the row, and the data are

based on all plants surviving at the time records were taken. Plots 3 and 4 were similarly planted to untreated and treated healthy seed at a double rate, and the seedlings were thinned when approximately 10 inches high so that the number and distribution of plants were identical with those in plots 1 and 2, respectively. In thinning, the seedlings were removed systematically according to position in order to maintain the normal proportion of inferior plants.

The comparisons between plots 1 and 3, showing the effect of Diplodia seed infection, and between 2 and 4, showing any remaining effect of the infection following seed treatment, may be made on identical but imperfect stand bases. A comparison between plots 1 and 2 shows the effect of treating Diplodia-infected seed where the progenies differ with respect to both disease and stand due to the treatment of seed in plot 2.

Plots 5, 6, 7, and 8 were duplicates, respectively, of 1, 2, 3, and 4 as to kind of seed planted, but differed in that all were planted at a double rate and thinned as closely as possible to uniform stands of one plant every 14 inches. The surplus plants, as in the previous case, were removed systematically according to their position in the hill in order to avoid changing the normal ratio of healthy and diseased seedlings. Further adjustment to perfect stand conditions was made by basing records on only those plants which were situated at least two spaces from skips within the row. Thus relatively uniform conditions were provided all plants except in so far as the effects of the vacant spaces both within the row and in adjacent rows were not entirely eliminated. These vacant space effects would be more pronounced in case of the diseased seed because of the greater number of skips.

After thinning, the interior rows to be used in obtaining data were assigned numbers and each plant was given an individual number. The position of each plant was so recorded that its development might be traced from the seedling stage to maturity and the determination of its individual yield.

The seedlings which were removed upon thinning the double-rate plots were considered representative of the respective populations from which they were drawn and were saved for determining individual moisture-free seedling weights. The weights were taken for the entire seedling less the roots.

Plant heights were determined at three stages of development. For the first two stages, five and nine weeks after planting, measurements were taken from the ground level to the tip of the longest leaf. When the plants were fully grown, the measurement was made to the lowest tassel branch.

The date of emergence of the first silks on each plant was determined by observing the plants daily. Any variability in time required to reach this stage could then be determined from these data.

Counts of bent and broken stalks, stalk and ear smut, barren plants, and suckers were made when the crop reached maturity.

The ears were harvested separately at the beginning of the normal husking season and assigned their individual plant numbers. Field weights were taken and the ears dried to a moisture-free basis in an oven. Weights were again taken for each ear, after which the ears were shelled and the weight of oven-dry shelled corn determined. Shrinkage and shelling percentages were then calculated.

CALCULATION OF BIOMETRICAL CONSTANTS

The biometrical constants used in the analysis of the data were obtained by the use of the following standard formulas:

$$\text{Mean} = M = GA + c \text{ (algebraic)}$$

$$c = \frac{\Sigma(fd) \text{ (algebraic)}}{N},$$

where GA = guessed average, c = correction of the guessed average, $\Sigma(fd)$ = summation of frequency × deviation from the guessed average, and N = number of variates.

$$\text{Standard deviation} = S.D. = \sqrt{\frac{\Sigma(fd)^2 - c^2}{N}}$$

$$\text{Coefficient of variability} = C.V. = \frac{S.D. \times 100}{M}$$

$$P.E. \text{ of mean} = \frac{\pm 0.6745\ S.D.}{\sqrt{N}}$$

$$P.E. \text{ of } S.D. = \frac{\pm 0.6745\ S.D.}{\sqrt{2N}}$$

$$P.E. \text{ of } C.V. \text{ for a } C.V. \text{ value of 10 or less} = \frac{\pm 0.6745\ C.V.}{\sqrt{2N}}$$

$$P.E. \text{ of } C.V. \text{ for a } C.V. \text{ value greater than 10} = \frac{\pm 0.6745\ C.V.}{\sqrt{2N}}\left[1 + 2\left(\frac{C.V.}{100}\right)^2\right]^{\frac{1}{2}}$$

$P.E.$ of a difference $= \sqrt{a^2 + b^2}$, where a and b represent the probable errors of the separate values compared.

In Tables 15 and 16 the deviation from the mean method described by Hayes and Garber (*1*) was used.

In the discussion of the results, a difference between two comparable constants must be at least 3.2 times its probable error to be considered as significant. This value of the ratio of the difference to its probable error corresponds to odds of approximately 30 to 1 that the difference is not due to error in the selection of the random sample. In appraising the results, it is well to remember that the value 3.2 is arbitrary.

EFFECT OF DIPLODIA ON THE VIABILITY OF THE SEED

The results of the laboratory germination test of the seed used in planting the plots are reported in Table 1. In reading the test, the kernels were classified into four groups according to the apparent vigor of the sprout, as follows: (1) "Strong," which included all kernels producing vigorous sprouts from unrotted kernels even though the kernel or sprout showed the presence of mold; (2) "weak," which included kernels that were partly rotted or that had distinctly less vigorous sprouts; (3) "dead sprouts," which included all kernels

producing sprouts that later had rotted; and (4) "dead kernels," which included all kernels that failed to germinate.

The total germination for diseased seed and healthy seed was, respectively, 72 and 91 per cent. The classification of each 100 kernels which germinated from the two lots of seed is also shown in Table 1. Of the kernels which germinated, diseased and healthy seed produced, respectively, 67 and 75 per cent strong sprouts. Likewise, of the germinating seeds, diseased seed produced 2 per cent more weak sprouts and 6 per cent more dead sprouts than did healthy seed.

TABLE 1.—*Laboratory germination test of Diplodia-infected seed and nearly disease-free seed used in the field experiments*

Character of germination	Germination percentages based on—			
	All seed planted		Viable seed	
	Diseased seed	Healthy seed	Diseased seed	Healthy seed
	Per cent	*Per cent*	*Per cent*	*Per cent*
Strong	48	68	67	75
Weak	16	18	22	20
Dead sprouts	8	5	11	5
Dead kernels	28	9	----------	----------
Total viable	72	91	----------	----------

EFFECT OF SEED INFECTION AND TREATMENT ON FIELD GERMINATION

The results of the observations of field germination are similar to those obtained in the laboratory, and are reported in Table 2. The final field stands 18 days after planting were: Diseased 52.4, treated diseased 71.1, healthy 89.0, and treated healthy 88.9 per cent. When compared with the results of the laboratory germination test, it will be observed that the 52.4 per cent field stand from the untreated diseased seed corresponds quite closely to the 48 per cent which germinated vigorously in the laboratory, while the 71.1 per cent field stand from treated diseased seed corresponds to the total of 72 per cent which germinated in the laboratory. The percentage of field stand from healthy seed, either untreated or treated, does not differ more than 2 per cent from the total germination in the laboratory.

The percentage of field stand was also determined at three dates previous to the time when emergence was considered as complete. The data in colums 7, 8, and 9, Table 2, show that the seedlings which did emerge from either untreated or treated diseased seed were not retarded, nor is there any evidence to show that the treatment of nearly disease-free seed stimulated emergence.

TABLE 2.—*Effects of Diplodia seed infection and of seed treatment upon the field germination of corn, determined 9, 12, 15, and 18 days after planting*

Kind of seed and treatment	Seeds planted	Percentage of seedling emergence on—				Percentage of the final emergence on—			
		May 25	May 28	May 31	June 3	May 25	May 28	May 31	June 3
Diseased, untreated	2,380	10.3	52.0	52.5	52.4	19.7	99.2	100.0	100.0
Diseased, treated	2,380	11.2	69.6	70.8	71.1	15.8	97.9	99.6	100.0
Healthy, untreated	3,400	14.6	87.2	89.1	89.0	16.4	97.8	100.0	100.0
Healthy, treated	3,400	14.4	87.2	88.5	88.9	16.2	98.1	99.6	100.0

EFFECT OF SEED INFECTION AND TREATMENT ON THE PERCENTAGE OF INFERIOR PLANTS

At the time of the first cultivation, five weeks after planting, the stands were examined for inferior plants. Plants which were spindling, stunted, off color, or otherwise lacking in vigor were considered as inferior. The results of this examination are shown in Table 3. The respective percentages of inferior plants from diseased seed, treated diseased seed, untreated healthy seed, and treated healthy seed were 11.9, 3.7, 2.7, and 2.5 per cent. While the treatment of diseased seed reduced the proportion of conspicuously inferior plants, there would seem to be no material advantage resulting from the treatment of healthy seed in reducing the percentage of such inferior plants. Of the inferior plants found in the seedling stage, 20 per cent disappeared before maturity.

TABLE 3.—*Effects of Diplodia seed infection and seed treatment upon the percentage of inferior corn plants, observed five weeks after planting, June 20, 1929*

Kind of seed and treatment	Total plants	Inferior plants	
	Number	*Number*	*Per cent*
Diseased, untreated	1,248	149	11.9
Diseased, treated	1,692	62	3.7
Healthy, untreated	3,025	83	2.7
Healthy, treated	3,023	76	2.5

FINAL RESULTS FROM INFERIOR PLANTS

Of the inferior plants noted in the seedling stage, 81 were so situated at maturity that they could be compared with adjacent normal plants. The results are reported in Table 4. In the seedling stage, the inferior

TABLE 4.—*Comparison of seedling height, mature height, and plant yield of inferior plants and adjacent normal plants, and all plants from which data were secured*

Kind of plants compared	Plants harvested	Average—		
		Seedling height	Mature height	Yield of shelled corn per plant
	Number	*Inches*	*Inches*	*Grams*
Inferior plants	81	10.0	58.1	74.7
Normal plants	81	19.7	71.4	158.9
All plants	[a] 3,417	20.3	68.6	129.4

[a] This number was slightly greater for the seedling heights due to loss of some plants by the time of maturity.

plants averaged 10 inches in height as compared with 19.7 inches for the adjacent normal plants. At maturity, the inferior plants averaged 58.1 inches, while the adjacent normal plants averaged 71.4 inches. The inferior plants averaged 74.7 gm. of shelled corn per plant as compared with 158.9 gm. for the adjacent normal plants, and 129.4 gm. as the mean for the entire population.

These data show that although the inferior plants were only 51 per cent as tall in the seedling stage as the normal plants, they were 81

per cent as tall at maturity. The individual grain yield of the inferior plants averaged 42 per cent less, while that of the adjacent normal plants averaged 23 per cent more than the mean yield of all the plants from which data were secured.

VARIABILITY OF PLANT CHARACTERS

In order to facilitate analysis of the comparisons under study, the description (p. 724) of the eight plantings upon which all data are based is shown in summary form in Table 5. For the sake of brevity,

TABLE 5.—*Kind of seed, treatment, planting rate, and kind of stand for the eight plantings on which all variability and other field data are based*

Plot No.	Number of replications	Kind of seed	Treatment	Rate of planting	Kind of stand
IMPERFECT STANDS					
1	10	Diseased	None	Single	Unadjusted.
2	10	do	Semesan Jr.	do	Do.
3	10	Healthy	None	Double	Adjusted identical to plot 1.
4	10	do	Semesan Jr.	do	Adjusted identical to plot 2.
NEARLY PERFECT STANDS					
5	10	Diseased	None	Double	Thinned to approximate perfect stand.
6	10	do	Semesan Jr.	do	Do.
7	10	Healthy	None	do	Do.
8	10	do	Semesan Jr.	do	Do.

the two types of stands typified by the two groups of plots, 1 to 4 and 5 to 8, will be referred to as imperfect and nearly perfect even though the latter is known to have been somewhat defective.

With the exception of Table 9, where the data for only four plots are given, Tables 6 to 13 show the mean results and their coefficients of variability for all eight plantings. The differences between certain of these means and coefficients are also given. The probable errors and the ratios D/PE are shown for these differences as accepted evidence of significance.

VARIABILITY IN SEEDLING HEIGHT AT AGE OF 5 WEEKS

In the imperfect stands (Table 6) the mean plant heights, five weeks after planting, for the progenies of diseased, treated diseased, healthy, and treated healthy seed were 17.85 ± 0.16, 19.77 ± 0.10, 20.63 ± 0.12, and 20.42 ± 0.08 inches, respectively, while the corresponding coefficients of variability were 28.63 ± 0.71, 17.35 ± 0.35, 17.66 ± 0.49, and 14.66 ± 0.29 per cent. Diplodia infection served to reduce the mean plant height 2.78 ± 0.20 inches and increased the variability 10.97 ± 0.86 per cent. The treatment of diseased seed increased the mean plant height 1.92 ± 0.19 inches and reduced the coefficient of variability 11.28 ± 0.79 per cent. Treatment of healthy seed resulted in an insignificant decrease of 0.21 ± 0.14 inch in plant height and a decrease of 3.0 ± 0.57 per cent in the coefficient of variability.

TABLE 6.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants, five weeks after planting, June 20, 1929*[a]

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants measured	Plant height	
			Mean	Coefficient of variability
		Number	*Inches*	
1	Diseased, untreated	428	17.85±0.16	28.63±0.71
2	Diseased, treated	598	19.77± .10	17.35± .35
3	Healthy, untreated	428	20.63± .12	17.66± .49
4	Healthy, treated	598	20.42± .08	14.66± .29

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated	246	18.37±0.21	26.05±0.82
6	Diseased, treated	447	21.41± .12	17.34± .42
7	Healthy, untreated	574	21.52± .10	16.27± .33
8	Healthy, treated	562	21.28± .09	14.30± .29

DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
	Inches			
Healthy, untreated (plot No. 3) minus diseased, untreated (Plot No. 1)	2.78±0.20	13.90	−10.97±0.86	12.76
Diseased, treated (plot No. 2) minus diseased, untreated (Plot No. 1)	1.92± .19	10.10	−11.28± .79	14.28
Healthy, treated (plot No. 4) minus healthy, untreated (Plot No. 3)	−.21± .14	1.50	−3.00± .57	5.26

DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (Plot No. 5)	3.15±0.23	13.70	−9.78±0.88	11.11
Diseased, treated (plot No. 6) minus diseased, untreated (Plot No. 5)	3.04± .24	12.67	−8.71± .92	9.47
Healthy, treated (plot No. 8) minus healthy, untreated (Plot No. 7)	−.24± .13	1.85	−1.97± .44	4.48
Healthy, untreated (plot No. 7) minus diseased, treated (Plot No. 6)	.11± .16	.69	−1.07± .53	2.02

[a] All plots were either 4 or 5 row plots 39 feet 8 inches long. The normal planting rate was one kernel every 14 inches in rows 42 inches apart. Plots 1 and 2 were planted at a normal rate. Plots 3 and 4 were planted at a double rate and thinned to the same number and distribution of plants, respectively, as grew in plots 1 and 2. In the case of plots 1 to 4, the data are based on all plants in the interior rows regardless of stand. In the case of plots 5 to 8, two seeds were dropped in the place of one, followed by thinning to one plant in a place. The variability studies were based on all plants in the interior rows which were removed two or more spaces from vacancies in the row.

For the plots containing a nearly perfect stand the mean heights of the progenies from diseased, treated diseased, healthy, and treated healthy seed were 18.37 ± 0.21, 21.41 ± 0.12, 21.52 ± 0.10, and 21.28 ± 0.09 inches. The corresponding coefficients of variability were 26.05 ± 0.82, 17.34 ± 0.42, 16.27 ± 0.33, and 14.30 ± 0.29 per cent. Diplodia infection resulted in a decrease of 3.15 ± 0.23 inches in mean plant height and an increase of 9.78 ± 0.88 per cent in coefficient of

variability. The treatment of diseased seed increased the mean plant height 3.04 ± 0.24 inches and reduced the coefficient of variability 8.71 ± 0.92 per cent. Treatment of healthy seed was accompanied by a reduction of 0.24 ± 0.13 inch in plant height and 1.97 ± 0.44 per cent in the coefficient of variability. Comparing the height of plants from treated diseased seed with that from untreated healthy seed, when grown under fairly comparable stand conditions at either this or later stages of development, no important difference was found.

VARIABILITY IN PLANT HEIGHT AT AGE OF 9 WEEKS

Under imperfect stand conditions (Table 7) the mean plant height at the age of 9 weeks for the progeny from diseased, treated diseased, healthy, and treated healthy seed were, respectively, 59.11 ± 0.32, 61.81 ± 0.22, 61.32 ± 0.24, and 60.81 ± 0.21 inches. Corresponding coefficients of variability were 16.48 ± 0.39, 12.52 ± 0.25, 11.59 ± 0.27, and 12.32 ± 0.25 per cent. As in the seedling stage, disease lowered the mean height and increased the variability. Likewise seed treatment increased the mean height and lowered the variability of the progeny from diseased seed. Treatment did not significantly affect the crop from healthy seed.

The effects of seed infection and seed treatment under nearly perfect stand conditions were almost identical with those just described.

TABLE 7.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants nine weeks after planting, July 16, 1929*[a]

MEAN HEIGHTS AND COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants measured	Plant height	
			Mean	Coefficient of variability
		Number	*Inches*	
1	Diseased, untreated	425	59.11±0.32	16.48±0.39
2	Diseased, treated	568	61.81± .22	12.52± .25
3	Healthy, untreated	412	61.32± .24	11.59± .27
4	Healthy, treated	585	60.81± .21	12.32± .25

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated	222	59.22±0.45	16.73±0.32
6	Diseased, treated	419	62.20± .26	12.70± .30
7	Healthy, untreated	531	63.51± .21	11.05± .23
8	Healthy, treated	534	63.38± .22	11.69± .25

DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
	Inches			
Healthy, untreated (Plot No. 3) minus diseased, untreated (Plot No. 1)	2.21±0.40	5.53	−4.89±0.47	10.40
Diseased, treated (Plot No. 2) minus diseased, untreated (Plot No. 1)	2.70+ .39	6.92	−3.96± .46	8.61
Healthy, treated (Plot No. 4) minus healthy, untreated (Plot No. 3)	−.51± .32	1.59	0.73± .37	1.97

[a] See footnote, Table 6, for details of planting.

TABLE 7.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants nine weeks after planting, July 16, 1929*—Continued

DIFFERENCES BETWEEN MEAN PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
	Inches			
Healthy, untreated (Plot No. 7) minus diseased, untreated (Plot No. 5)	4.29±0.50	8.58	−5.68±0.39	14.56
Diseased, treated (Plot No. 6) minus diseased, untreated (Plot No. 5)	2.98± .52	5.73	−4.03± .44	9.16
Healthy, treated (Plot No. 8) minus healthy, untreated (Plot No. 7)	−.13± .30	.43	.64± .34	1.88
Healthy, untreated (Plot No. 7) minus diseased, treated (Plot No. 6)	1.31± .33	3.97	−1.65± .38	4.34

VARIABILITY IN PLANT HEIGHT AT MATURITY

At maturity, under imperfect stand conditions (Table 8) the mean plant heights to the lowest tassel branch for progenies of diseased, treated diseased, healthy, and treated healthy seed were 67.57 ± 0.31, 67.50 ± 0.23, 67.92 ± 0.27, and 68.45 ± 0.24 inches, respectively. Corresponding coefficients of variability were 13.35 ± 0.29, 12.00 ± 0.25, 11.76 ± 0.28, and 12.44 ± 0.25 per cent. Diplodia infection reduced the mean plant height 0.35 ± 0.41 inch and increased the coefficient of variability 1.59 ± 0.40 per cent. The treatment of diseased seed lowered the mean plant height only 0.07 ± 0.39 inch and lowered the coefficient of variability 1.35 ± 0.38 per cent. These data indicate no significant effect from Diplodia or from treatment of either diseased or healthy seed upon the mean height, and only very slight effects upon the variability.

TABLE 8.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants at maturity* [a]

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants measured	Plant height	
			Mean	Coefficient of variability
		Number	*Inches*	
1	Diseased, untreated	397	67.57±0.31	13.35±0.29
2	Diseased, treated	560	67.50± .23	12.00± .25
3	Healthy, untreated	412	67.92± .27	11.76± .28
4	Healthy, treated	568	68.45± .24	12.44± .25

MEAN HEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated	210	67.93±0.42	13.26±0.44
6	Diseased, treated	383	69.15± .28	11.78± .29
7	Healthy, untreated	507	70.11± .26	12.50± .27
8	Healthy, treated	499	69.63± .25	12.01± .26

[a] See footnote, Table 6, for details of planting.

TABLE 8.—*Effects of Diplodia seed infection and seed treatment upon the mean height and variability of corn plants at maturity*—Continued

DIFFERENCES BETWEEN MEAN MATURE PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Plant height			
	Mean	D/PE	Coefficient of variability	D/PE
	Inches			
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1)	0.35±0.41	0.85	−1.59±0.40	3.98
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1)	−.07± .39	.18	−1.35± .38	3.55
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3)	.53± .36	1.47	−.68± .38	1.79

DIFFERENCES BETWEEN MEAN MATURE PLANT HEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	2.18±0.49	4.45	−0.76±0.52	1.46
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	1.22± .50	2.44	−1.48± .53	2.79
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7)	−.48± .36	1.33	−.49± .37	1.32
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	.96± .38	2.53	.72± .40	1.80

In the presence of a more nearly perfect stand (plots 5 to 8) the mean heights of plants at maturity were 67.93 ± 0.42, 69.15 ± 0.28, 70.11 ± 0.26, and 69.63 ± 0.25 inches, respectively, from the planting of diseased, treated diseased, healthy, and treated healthy seed. The corresponding coefficients of variability were 13.26 ± 0.44, 11.78 ± 0.29, 12.50 ± 0.27, and 12.01 ± 0.26 per cent. Thus Diplodia lowered the mean height 2.18 ± 0.49 inches but had no significant effect upon variability. Treatment increased the mean plant height for diseased seed 1.22 ± 0.50 inches and lowered the coefficient of variability 1.48 ± 0.53 per cent.

Taking all of these data into consideration, it appears that both disease and treatment have had an almost negligible effect upon the mature progeny of diplodia-infected seed. The rather pronounced differences in variability in the seedling stage have been largely outgrown. Treating healthy seed had no significant effects.

VARIABILITY IN MOISTURE-FREE SEEDLING WEIGHT

The mean moisture-free weights of seedlings grown from diseased (Table 9), treated diseased, healthy, and treated healthy seed were 1.72 ± 0.047, 1.96 ± 0.039, 1.94 ± 0.028, and 2.00 ± 0.026 gm., respectively. Corresponding coefficients of variability were 52.73 ± 2.39, 51.57 ± 1.73, 42.46 ± 1.17, and 42.04 ± 1.08 per cent. Diplodia infection reduced the mean seedling weight 0.22 ± 0.055 gm. and raised the variability 10.27 ± 2.66 per cent. The treatment of diseased seed increased the mean seedling weight 0.24 ± 0.061 gm. but did not materially affect the coefficient of variability. Treatment had no significant effect on the progeny from healthy seed.

TABLE 9.—*Effects of Diplodia seed infection and seed treatment upon the mean moisture-free seedling weight of corn and its coefficient of variability, when weighings were made four weeks after planting, June 17, 1929*

MEAN WEIGHTS AND THEIR COEFFICIENTS OF VARIABILITY

Plot No.	Kind of seed and treatment [a]	Plants weighed	Seedling weight	
			Mean	Coefficient of variability
		Number	*Grams*	
5	Diseased, untreated	172	1.72±0.047	52.73±2.39
6	Diseased, treated	310	1.96± .039	51.57±1.73
7	Healthy, untreated	409	1.94± .028	42.46±1.17
8	Healthy, treated	470	2.00± .026	42.04±1.08

DIFFERENCES BETWEEN MEAN WEIGHTS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY

Kind of seed and treatment	Oven-dry seedling weights			
	Mean	D/PE	Coefficient of variability	D/PE
	Grams			
Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	0.22±0.055	4.00	−10.27±2.66	3.86
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	.24± .061	3.93	−1.16±2.95	.39
Healthy, treated (plot No. 8) minus Healthy, untreated (plot No. 7)	.06± .038	1.58	−.42±1.59	.26
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	−.02± .048	.42	−9.11±2.09	4.36

[a] The seedlings used in this test were systematically removed, at the time of thinning, from plots 5 to 8 which had been planted at a double rate.

VARIABILITY IN NUMBER OF DAYS FROM PLANTING TO SILKING

The mean number of days from planting to silking and the coefficients of variability for each of the eight plantings are shown in Table 10. The maximum difference in length of time required to reach the fruiting period did not exceed one day, and there were no important differences in variability. In no case did either Diplodia seed infection or seed treatment influence the mean silking period more than 0.7 day nor did differences in the coefficients of variability exceed 1 per cent. Most of the differences showed very slight significance.

TABLE 10.—*Effects of Diplodia seed infection and seed treatment upon the mean number of days between planting and silking of corn and its coefficient of variability* [a]

MEAN NUMBERS OF DAYS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants observed	Period to silking	
			Mean	Coefficient of variability
		Number	*Days*	
1	Diseased, untreated	395	75.03±0.12	4.85±0.12
2	Diseased, treated	551	74.98± .10	4.69± .10
3	Healthy, untreated	406	74.59± .10	4.17± .10
4	Healthy, treated	558	75.29± .08	3.88± .08

[a] See footnote, Table 6, for details of planting.

TABLE 10.—*Effects of Diplodia seed infection and seed treatment upon the mean number of days between planting and silking of corn and its coefficient of variability*—Continued

MEAN NUMBERS OF DAYS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Plot No.	Kind of seed and treatment	Plants observed	Period to silking	
			Mean	Coefficient of variability
		Number	*Days*	
5	Diseased, untreated	213	75.89±0.18	5.19±0.17
6	Diseased, treated	391	75.58± .12	4.50± .11
7	Healthy, untreated	501	75.61± .11	4.82± .10
8	Healthy, treated	494	75.89± .10	4.44± .10

DIFFERENCES BETWEEN MEAN NUMBERS OF DAYS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Period to silking			
	Mean	D/PE	Coefficient of variability	D/PE
	Days			
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No 1)	−0.44±0.16	2.75	−0.68±0.16	4.25
Diseased, treated (plot No. 2) minus diseased, treated (plot No. 1)	−.05± .16	.31	−.16± .16	1.00
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3)	.70± .13	5.38	−.20± .13	2.23

DIFFERENCES BETWEEN MEAN NUMBERS OF DAYS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	−0.28±0.21	1.33	−0.37±0.20	1.85
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	−.31± .22	1.41	−.69± .20	3.45
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7)	.28± .15	1.87	−.38± .14	2.71
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	.03± .16	.19	.32± .15	2.13

VARIABILITY IN THE SHRINKAGE OF EAR CORN

Shrinkage determinations are of interest as suggesting comparative maturity of the crop. In the first four plots which were subject to imperfect stands (Table 11) the mean ear-corn shrinkage percentages for diseased, treated diseased, healthy, and treated healthy seed were 28.44 ± 0.32, 26.64 ± 0.25, 28.11 ± 0.27, and 26.81 ± 0.25 per cent, respectively. The corresponding coefficients of variability were 32.34 ± 0.86, 32.95 ± 0.74, 28.71 ± 0.73, and 32.92 ± 0.73 per cent. Diplodia seed infection insignificantly increased the shrinkage 0.33 ± 0.42 per cent, while the treatment of diseased seed lowered the shrinkage 1.80 ± 0.41 per cent.

Under uniform stand conditions, the mean shrinkage percentages were 26.88 ± 0.50, 25.27 ± 0.29, 26.27 ± 0.32, and 26.48 ± 0.33 per cent, respectively, for plots 5 to 8. Associated coefficients of variability were 39.66 ± 1.51, 33.12 ± 0.90, 39.44 ± 0.98, and 39.65 ± 1.00 per cent. Seed infection insignificantly increased the shrinkage 0.61 ± 0.59 per cent, while treatment of diseased seed lowered the shrinkage 1.61 ± 0.58 per cent. No differences in variability were statistically significant except where the treated diseased seed was involved. The variability of this planting was so outstandingly low as to suggest an abnormal result.

TABLE 11.—*Effects of Diplodia seed infection and seed treatment upon the mean shrinkage percentage of ear corn and its coefficient of variability* [a]

MEAN SHRINKAGE PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Ears weighed	Shrinkage	
			Mean	Coefficient of variability
		Number	*Per cent*	
1	Diseased, untreated	392	28.44±0.32	32.34±0.86
2	Diseased, treated	547	26.64± .25	32.95± .74
3	Healthy, untreated	405	28.11± .27	28.71± .73
4	Healthy, treated	559	26.81± .25	32.92± .73

MEAN SHRINKAGE PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated	207	26.88±0.50	39.66±1.51
6	Diseased, treated	374	25.27± .29	33.12± .90
7	Healthy, untreated	482	26.27± .32	39.44± .98
8	Healthy, treated	474	26.48± .33	39.65±1.00

DIFFERENCES BETWEEN MEAN SHRINKAGE PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Shrinkage			
	Mean	D/PE	Coefficient of variability	D/PE
	Per cent			
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1)	−0.33±0.42	0.79	−3.63±1.13	3.21
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1)	−1.80± .41	4.39	− .61±1.13	.54
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3)	−1.30± .37	3.51	4.21±1.03	4.09

DIFFERENCES BETWEEN MEAN SHRINKAGE PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	−0.61±0.59	1.03	−0.22±1.80	0.12
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	−1.61± .58	2.78	−6.54±1.76	3.72
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7)	.21± .46	.46	.21±1.40	.15
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	1.00± .43	2.33	6.32±1.33	4.75

[a] See footnote, Table 6, for details of planting.

VARIABILITY IN THE SHELLING PERCENTAGE OF EAR CORN

The data in Table 12 indicate that neither Diplodia infection nor the treatment of either diseased or healthy seed had any significant effect on the mean percentage of shelled corn. The mean shelling percentages for imperfect stand conditions were, respectively, 80.95 ± 0.26, 80.59 ± 0.20, 81.19 ± 0.20, and 81.38 ± 0.17 for diseased, treated diseased, healthy, and treated healthy seed. The corresponding coefficients of variability were 8.58 ± 0.19, 8.39 ± 0.17, 7.24 ± 0.17, and 7.31 ± 0.15 per cent, respectively. The ears from diseased seed, either untreated or treated, were slightly more variable as to shelling percentage than those from healthy seed.

TABLE 12.—*Effects of Diplodia seed infection and seed treatment upon the mean shelling percentage of ear corn and its coefficient of variability* [a]

MEAN SHELLING PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Ears weighed	Shelling per cent	
			Mean	Coefficient of variability
		Number		
1	Diseased, untreated	392	80.95±0.26	8.58±0.19
2	Diseased, treated	544	80.59± .20	8.39± .17
3	Healthy, untreated	404	81.19± .20	7.24± .17
4	Healthy, treated	558	81.38± .17	7.31± .15

MEAN SHELLING PERCENTAGES AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated	204	80.47±0.33	8.75±0.29
6	Diseased, treated	374	80.91± .23	8.08± .20
7	Healthy, untreated	480	80.03± .26	10.36± .23
8	Healthy, treated	472	80.49± .24	9.71± .21

DIFFERENCES BETWEEN MEAN SHELLING PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Shelling per cent			
	Mean	D/PE	Coefficient of variability	D/PE
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1)	0.24±0.33	0.73	−1.34±0.25	5.36
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1)	−.36± .33	1.09	−.19± .25	.76
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3)	.19± .26	.73	−.07± .23	.30

DIFFERENCES BETWEEN MEAN SHELLING PERCENTAGES AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	−0.44±0.42	1.05	1.61±0.37	4.35
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	.44± .40	1.10	−.67± .35	1.91
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7)	.46± .35	1.31	−.65± .31	2.10
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	−.88± .35	2.51	2.28± .30	7.60

[a] See footnote, Table 6, for details of planting.

In the case of the plots which approximated a full stand of plants, the mean shelling percentages of ears grown from diseased, treated diseased, healthy, and treated healthy seed were 80.47 ± 0.33, 80.91 ± 0.23, 80.03 ± 0.26, and 80.49 ± 0.24 per cent, respectively. As was true of the imperfect stands, these means do not differ significantly from each other. The coefficients of variability for the same plots were 8.75 ± 0.29, 8.08 ± 0.20, 10.36 ± 0.23, and 9.71 ± 0.21 per cent,

respectively. The ears grown from either untreated or treated healthy seed were significantly less uniform in shelling percentage than ears grown from corresponding diseased seed. There seems to be no explanation why ears from the more uniform stands should be more variable in shelling percentage than those from the less uniform stands. When the corresponding coefficients of variability were averaged for both kinds of stand, they were found to be 8.67 ± 0.17, 8.24 ± 0.13, 8.80 ± 0.14, and 8.51 ± 0.13 per cent, respectively, for ears produced from the diseased, treated diseased, healthy, and treated healthy seed. If it may be assumed that these averages are reliable, then there would appear to be no real difference in the variability of the shelling percentages from the various lots of seed.

VARIABILITY IN THE YIELD OF GRAIN PER PLANT

The mean oven-dry yields of shelled corn per plant are reported in Table 13. Plants grown under imperfect stand conditions from diseased, treated diseased, healthy, and treated healthy seed produced the respective mean grain yields of 158.43 ± 2.12, 134.43 ± 1.35, 159.98 ± 1.88, and 136.55 ± 1.40 gm. These results show a 1 per cent lower mean yield per plant from heavily Diplodia-infected seed than was obtained from almost identically distributed plants grown from healthy seed. This difference is not significant since the probable error is nearly twice the difference. Treatment of the diseased seed resulted in an increased stand at maturity of 39 per cent and a decrease in mean yield per plant of 15 per cent. This decrease was no doubt due to the increased competition in the plots having the thicker stand.

TABLE 13.—*Effects of Diplodia seed infection and seed treatment upon the mean yield of shelled corn per plant and its coefficient of variability*[a]

MEAN YIELDS AND THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Plot No.	Kind of seed and treatment	Plants	Shelled corn per plant	
			Mean	Coefficient of variability
		Number	*Grams*	
1	Diseased, untreated	391	158.43±2.12	39.14±1.25
2	Diseased, treated	544	134.43±1.35	34.60± .76
3	Healthy, untreated	404	159.98±1.88	35.08± .93
4	Healthy, treated	558	136.55±1.40	35.86± .81

MEAN YIELDS AND THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

5	Diseased, untreated	204	131.86±2.29	36.80±1.39
6	Diseased, treated	374	111.80±1.51	38.64±1.09
7	Healthy, untreated	470	105.50±1.37	41.68±1.07
8	Healthy, treated	472	101.31±1.23	38.86± .97

[a] See footnote, Table 6, for details of planting.

TABLE 13.—*Effects of Diplodia seed infection and seed treatment upon the mean yield of shelled corn per plant and its coefficient of variability*—Con.

DIFFERENCES BETWEEN MEAN YIELDS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR IMPERFECT STANDS

Kind of seed and treatment	Shelled corn per plant			
	Mean	D/PE	Coefficient of variability	D/PE
	Grams			
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1)	1.55±2.83	0.55	−4.06±1.56	2.60
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1)	−24.00±2.51	9.56	−4.54±1.46	3.11
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3)	−23.43±2.34	10.01	.78±1.23	.63

DIFFERENCES BETWEEN MEAN YIELDS AND BETWEEN THEIR COEFFICIENTS OF VARIABILITY FOR NEARLY PERFECT STANDS

Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	−26.36±2.67	9.87	4.88±1.75	2.79
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	−20.06±2.74	7.32	1.84±1.77	1.04
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7)	−4.19±1.84	2.28	−2.82±1.44	1.96
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	−6.30±2.04	3.09	3.04±1.53	1.99

In the case of the adjusted stands of 44.9 per cent from untreated healthy seed and 62.0 per cent from treated healthy seed, the 38 per cent greater stand resulted in a decreased mean plant yield of 15 per cent. These data indicate that under conditions of less than optimum stand, changes in the number of plants per acre are accompanied by somewhat smaller inverse changes in the yield of individual plants. Since this holds true in about equal measure for both diseased and healthy seed, it seems probable that increased yields per acre resulting from seed treatment are largely due to stand improvement.

MISCELLANEOUS FIELD DATA

Individual plant notes concerning various plant characters of agronomic interest were taken on all plants involved in these studies. These are summarized in Table 14. It is evident that neither seed infection nor treatment very materially influenced the percentage of bent, broken, barren, or 2-eared stalks. Variations in the number of plants having either moldy or smutty ears did not exceed 1 per cent. While the variation in number of smutty stalks was as great as for any of the plant characters observed, the life history of the smut disease will not permit ascribing this to the effects of either Diplodia or treatment. The treatment of both diseased and healthy seed was accompanied by a somewhat lower percentage of plants developing suckers. Taking all of these data into consideration, it is not apparent how any of these miscellaneous effects could have economic importance.

TABLE 14.—*Effects of Diplodia seed infection and seed treatment upon various plant characters of corn*

Kind of seed and treatment	Population	Number of plants per hundred—							
		Bent	Broken	Smutted in—		Having suckers	Barren	Bearing 2-eared stalks	Bearing moldy ears
				Ear	Stalk				
	Number								
Diseased, untreated	607	4.9	1.5	0.4	8.4	15.2	2.6	4.4	1.0
Diseased, treated	943	7.0	2.4	.9	7.8	10.5	2.4	2.3	.7
Healthy, untreated	919	7.2	2.4	.8	12.0	20.7	3.7	3.3	.9
Healthy, treated	1,067	8.1	2.4	.5	10.4	14.9	2.4	2.7	.4

EFFECT OF DISEASE AND TREATMENT OF SEED ON ACRE YIELDS

The yields of grain have been determined on an acre basis for the various lots of seed tested under both conditions of stand. The comparative results are reported in Tables 15 and 16. The yields are calculated for the interior rows of the 4-row or 5-row plots without corrections for missing plants, and are averages of 10 systematic replications. The probable errors are indicated for the mean yields and for the differences between yields in certain important comparisons.

Under the conditions of a standard planting rate, the diseased and treated diseased seed produced stands of 43.4 and 60.4 per cent, respectively, and the corresponding yields were 34.0 ± 0.59 and 39.5 ± 0.68 bushels.

Under conditions of comparable stands adjusted to be essentially identical with those above, stands of 44.9 and 62.0 per cent from healthy and treated healthy seed yielded 35.0 ± 0.61 and 41.2 ± 0.71 bushels. Thus diseased seed producing a stand of 43.4 per cent yielded 34.0 ± 0.59 bushels, while healthy seed adjusted to a comparable stand of 44.9 per cent yielded 35.0 ± 0.61 bushels. This difference of 1.0 ± 0.9 bushel is only 1.11 times the probable error and is not to be regarded as significant.

TABLE 15.—*Effects of Diplodia seed infection and seed treatment upon the yield of shelled corn per acre*

[The yields reported are for all plants in plots 1 to 4 and are based on the entire area of the plots]

MEAN YIELDS [a] PER ACRE

Plot No.	Kind of seed and treatment	Stand	Mean yield per acre
		Per cent	*Bushels*
1	Diseased, untreated	43.4	34.0±0.59
2	Diseased, treated	60.4	39.5± .68
3	Healthy, untreated	44.9	35.0± .61
4	Healthy, treated	62.0	41.2± .71

DIFFERENCE BETWEEN MEANS

Kind of seed and treatment	Mean	D/PE
	Bushels	
Healthy, untreated (plot No. 3) minus diseased, untreated (plot No. 1)	1.0±0.9	1.11
Diseased, treated (plot No. 2) minus diseased, untreated (plot No. 1)	5.5± .9	6.11
Healthy, treated (plot No. 4) minus healthy, untreated (plot No. 3)	6.2± .9	6.89

[a] Yields in bushels per acre are reported on a basis of 14 per cent moisture content.

TABLE 16.—*Effects of Diplodia seed infection and seed treatment upon the yield of shelled corn per acre*

[The yields reported are for all the plants in the double-rate thinned plots and are based on the entire area of the plots]

MEAN YIELDS [a] PER ACRE

Plot No.	Kind of seed and treatment	Stand	Mean yield per acre
		Per cent	*Bushels*
5	Diseased, untreated	68.3	45.0±0.66
6	Diseased, treated	93.0	47.5± .69
7	Healthy, untreated	89.5	47.0± .69
8	Healthy, treated	89.8	45.6± .67

DIFFERENCE BETWEEN MEANS

Kind of seed and treatment	Mean	D/PE
	Bushels	
Healthy, untreated (plot No. 7) minus diseased, untreated (plot No. 5)	2.0±.95	2.11
Diseased, treated (plot No. 6) minus diseased, untreated (plot No. 5)	2.5±.95	2.63
Healthy, treated (plot No. 8) minus healthy, untreated (plot No. 7)	−1.4±.96	1.46
Healthy, untreated (plot No. 7) minus diseased, treated (plot No. 6)	−.5±.98	.51

[a] Yields in bushels per acre are reported on a basis of 14 per cent moisture content.

The treatment of diseased seed gave an increased stand of 39 per cent and an increased yield per acre of 16 per cent, while the treatment of healthy seed adjusted to an increased stand of 38 per cent gave an increased yield of 18 per cent.

The 5.5 bushels per acre increase due to the treatment of diseased seed is 6.11 times the probable error and is therefore to be regarded as a significant difference. The difference of 6.2 bushels in the yield from untreated healthy and treated healthy seed is 6.89 times the probable error.

Since the treatment of diseased and healthy seed, adjusted to equal stands, was accompanied by almost equal yield increases, it is evident that the increase in case of the diseased seed is due to the greater number of plants rather than to a greater mean yield from the individual plants.

In case of the plots in which an effort was made to approach a perfect stand by thick planting and thinning, there were no significant differences between any of the yields. The diseased, treated diseased, healthy, and treated healthy seed produced respectively, 45.0 ± 0.66, 47.5 ± 0.69, 47.0 ± 0.69, and 45.6 ± 0.67 bushels per acre. Corresponding field stands were 68.3, 93.0, 89.5, and 89.8 per cent at maturity. With a 36 per cent greater stand in the case of treated as compared with untreated diseased seed, there was only the rather small difference of 2.5 ± 0.95 bushels in yield. Where the basic stand was thinner, as in the case of plots 1 and 2, an almost identical percentage increase in stand resulted in a much greater and more significant yield increase.

EFFECT OF TREATING FARM-SELECTED SEED

The foregoing tests have been concerned only with comparisons of seed corn which had been specifically selected on the one hand because of heavy natural infection with *Diplodia zeae* and on the other hand for freedom from such disease organisms. Neither extreme represented such seed as farmers pick for planting by ordinary selection methods. While four years' results from experiment station tests with farm-selected seed (Kiesselbach (*3*)) have been published, the additional data secured in 1929 with such seed are of interest by way of substantiating the earlier conclusions. Ten lots of seed obtained from Nebraska corn growers and eight lots from growers in other States were planted comparably, both with and without treatment, in three replicated series of field plots. The plots contained three rows of 15 hills each, spaced 42 inches apart. Planting was done by hand at the rate of three seeds per hill. Treatment consisted of thorough dusting with Semesan Jr. The crop harvested was stored until uniformly air dry, when it was shelled and grain yields were calculated. Detailed observations concerning plant development were also recorded for all plots. These together with the yields are summarized in Table 17.

TABLE 17.—*Effects of seed treatment upon the development and grain yield from farm-selected seed corn obtained from 18 growers in Nebraska and other States, 1929*

SEED FROM NEBRASKA GROWERS

Variety	Seed source		Seed treatment	Field stand in—		Date in tassel	Mature height	Percentage of plants—				Inferior plants in—		Moldy ears	Shrinkage of ear corn	Yield of shelled corn per acre
	Grower	State or locality		Spring	Fall			Barren	Lodged or broken	With suckers	Smutty	Spring	Fall			
				Per cent	*Per cent*	*July*	*Inches*					*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Bushe*
Hogue	Experiment station.	Nebraska: Lincoln	Treated	90	89	27	88	10	52	28	3	6	2	1	8	46.8
			None	89	89	28	89	12	48	41	9	8	2	0	6	48.4
Do	Booth	Utica	Treated	97	87	28	92	9	67	24	9	2	4	0	8	45.9
			None	95	92	29	91	4	76	19	10	4	4	2	9	48.1
Reid	Aye	Blair	Treated	95	91	28	97	14	65	10	12	2	1	0	6	40.5
			None	94	92	29	98	15	57	13	11	4	2	1	9	45.3
Do	Roggenback	Wisner	Treated	99	91	25	95	21	46	9	19	5	4	0	6	37.2
			None	95	87	25	94	30	44	6	21	4	4	1	5	39.3
Do	Wahlgren	Elk City	Treated	98	92	25	92	17	57	7	12	2	2	0	7	37.3
			None	98	91	25	92	21	45	12	19	2	4	1	5	39.0
Do	Gramlich	Papillion	Treated	95	87	31	92	19	59	12	13	4	4	1	9	36.9
			None	90	87	31	90	20	58	10	12	6	3	1	10	35.5
St. Charles	Ward	Lincoln	Treated	97	92	25	90	10	61	10	13	3	2	1	10	42.4
			None	97	87	24	88	9	54	4	16	5	4	1	9	41.9
Gold Mine	Erskine	Havelock	Treated	93	91	26	93	13	62	11	9	6	3	0	6	40.1
			None	87	86	25	96	10	55	18	10	7	4	0	6	40.4
Kuhn	Sonderegger	Beatrice	Treated	97	92	26	86	16	60	17	6	3	4	1	11	43.2
			None	96	91	26	89	19	62	27	10	3	4	1	11	45.2
White	Substation	North Platte	Treated	99	89	23	83	11	43	8	11	4	5	3	4	37.8
			None	99	92	23	80	11	51	6	13	3	2	1	2	37.9
Average			Treated	96	90	26	91	14	57	14	11	4	3	1	8	40.8
			None	94	89	26	91	15	55	16	13	5	3	1	7	42.1

TABLE 17.—*Effects of seed treatment upon the development and grain yield from farm-selected seed corn obtained from 18 growers in Nebraska and other States, 1929*—Continued

SEED FROM GROWERS IN OTHER STATES

Variety	Seed source		Seed treatment	Field stand in—		Date in tassel	Mature height	Percentage of plants—				Inferior plants in—		Moldy ears	Shrinkage of ear corn	Yield of shelled corn per acre
	Grower	State or locality		Spring	Fall			Barren	Lodged or broken	With suckers	Smutty	Spring	Fall			
				Per cent	*Per cent*	*July*	*Inches*					*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Bushel*
Krug	Bebensee	Iowa	Treated	98	92	30	92	11	55	3	14	3	3	1	4	45.0
			None	94	87	30	92	17	63	8	10	4	2	0	8	43.3
Do	Pfister	Illinois	Treated	92	85	27	91	19	62	3	17	4	1	0	4	45.9
			None	93	87	26	92	13	51	8	16	5	4	0	6	44.7
Reid	Wilson	Iowa	Treated	93	92	26	90	13	58	6	13	3	2	0	7	41.2
			None	94	92	26	89	13	55	12	14	3	6	1	7	46.0
Colby	do	Kansas	Treated	92	92	28	83	8	56	8	11	4	3	2	5	47.8
			None	89	87	28	83	13	56	1	11	3	3	0	5	42.5
Reid	Carter	Illinois	Treated	88	82	26	90	13	44	6	16	4	3	1	3	36.4
			None	85	79	26	89	15	40	6	19	5	3	1	5	36.5
Ioleaming	Smith	Iowa	Treated	87	87	22	86	18	38	14	15	3	3	0	2	32.7
			None	92	89	22	86	18	31	10	8	3	3	0	1	34.5
Hi-Bred	Newlin	do	Treated	97	92	27	92	9	33	6	11	2	2	1	0	43.5
			None	94	88	27	90	7	36	5	11	3	4	3	0	45.0
Murdock		Minnesota	Treated	82	82	20	82	23	63	9	14	7	6	0	7	26.1
			None	78	78	20	81	16	49	2	14	5	3	0	5	24.6
Average			Treated	91	88	26	88	14	51	7	14	4	3	1	4	39.8
			None	90	86	26	88	14	48	7	13	4	4	1	5	39.6
Grand average			Treated	94	89	26	90	14	54	11	12	4	3	1	6	40.4
			None	92	88	26	89	14	52	12	13	4	3	1	6	41.0

While there were some differences among the various samples with respect to their reaction to seed treatment, due perhaps in part to chance variation, there was no very material effect on an average for all of the samples. Identical results were obtained for time of tasseling and ripening, plant height, percentage of inferior plants four weeks after planting and also at maturity, percentage of barren plants, moldy ears, shrinkage of ear corn, and shelling percentage. Slight but unimportant differences occurred in the percentage of plants affected with tillers, smut, and lodging. The field count showed 2 per cent more plants from treated seed in the spring and 1 per cent more in the fall than were obtained from untreated seed. The yields of air-dry shelled grain per acre without correction for missing plants were 41 bushels for untreated seed and 40.4 bushels for treated seed. This is an average difference of 0.6 bushel in favor of no treatment. These data confirm the negative results obtained at this station in other years.

EFFECT OF TREATING VARIOUS GRADES OF SEED CORN DURING A PERIOD OF YEARS

Three years of seed-corn-treatment tests with Diplodia-infected and nearly disease-free seed corn have now been completed at the Nebraska experiment station. In addition, various representative collections of farm-selected or planter-box seed have been tested for their response to disinfection during a 5-year period. Since all of the results obtained prior to 1929 have been published (*3*, *4*), a mere summary of field stands and acre yields will be included here. The treatments in any year have consisted of one or more of the standard commercial organic mercury seed disinfectants. The number of farmer's samples has varied from 6 to 30 annually. The selected diseased and healthy seed have been of the same Illinois variety or hybrid in any given year, and was procured for these tests from J. R. Holbert, of the United States Department of Agriculture. The test plots have consisted of three or four rows with three seeds dropped in hills 3½ feet apart. Yields were based on the interior rows.

TABLE 18.—*Summary of seed-corn-treatment tests with Diplodia-infected, selected healthy, and farm-selected seed corn during a period of years, 1925–1929* [a]

PERCENTAGE OF FIELD STAND OBTAINED

Year	Percentage stand obtained from seed that was—					
	Diplodia infected		Nearly disease free		Farm selected	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
1925					79	79
1926					73	73
1927	80	89	95	95	87	85
1928	69	78	93	91	96	95
1929	65	76	95	94	89	90
Average for three years	68	81	94	93	91	90
Average for five years					85	85

[a] The tests with diseased and healthy seed were strictly comparable since germinator selected seed grown at Bloomington, Ill., was procured from J. R. Holbert of the U. S. Department of Agriculture each year. The source and number of samples of Nebraska farm-selected seed varied from year to year. The number of farmers whose corn was represented were as follows: 1925, 30; 1926, 30; 1927, 17; 1928, 6; 1929, 10.

TABLE 18.—*Summary of seed-corn-treatment tests with Diplodia-infected, selected healthy, and farm-selected seed corn during a period of years, 1925–1929*—Con.

YIELD PER ACRE IN BUSHELS OF AIR-DRY SHELLED CORN

1925					39.4	39.1
1926					7.2	7.2
1927	57.9	62.2	64.7	66.7	55.0	53.9
1928	27.4	28.1	25.6	24.8	27.5	27.5
1929	35.1	43.5	41.1	40.2	42.1	40.8
Average for three years	40.1	44.6	43.8	43.9	41.5	40.7
Average for five years					34.2	33.7

The results reported in Table 18 indicate that during three years untreated healthy seed surpassed the untreated diseased seed 38 per cent (fig. 1) in the number of plants produced and 3.7 bushels, or 9.2 per cent, in the yield of grain per acre.

FIGURE 1.—Comparative development of the progenies from healthy (right) and Diplodia-infected (left) seed corn. While the diseased seed resulted in a much thinner stand and somewhat more variable population, the inferiority was scarcely apparent through a mere inspection from the end of the plots because a large percentage of the plants were fully normal

Seed treatment increased the stand of the diseased seed 19 per cent and the grain yield 4.5 bushels per acre. Neither the healthy seed nor the farm-selected seed responded favorably to seed disinfection.

DISCUSSION

The results with germinator-selected Diplodia-infected and healthy seed, farm-selected seed, and seed treatment reported herein for the year 1929 conform with those of similar tests in other years. Climatic conditions were fairly normal during the growing season of 1929, and it seems probable that crop responses to seed infection and seed treatment would average approximately the same in further tests. Based on the records of the United States Weather Bureau at Lincoln, the mean temperatures for May, June, July, and August, 1929, were 60.0°, 70.4°, 77.4°, and 76.2° F., respectively. These were departures of −1.7°, −1.0°, 0.9°, and 1.8° from the normal means of the respective months. Corresponding precipitation during these four months was 3.28, 1.86, 5.32, and 3.65 inches, respectively. These

represent departures of −0.80, −2.46, 1.47, and −0.06 inches from the normal.

In these tests the effects of seed-infection with Diplodia were determined through a comparison of plants from infected seed with plants from healthy seed that had been selected for relative freedom from dry-rot organisms. The two lots of seed differed distinctly with respect to germinative power. In laboratory tests only 72 per cent of the diseased seed proved viable, and in the field only 52 per cent emerged and produced seedlings. Ninety-one per cent of the healthy seed was found viable and 89 per cent of a field stand was obtained. Disinfection of the diseased seed increased the field stand by 36 per cent, while it did not affect the stand from healthy seed. These data show that while the healthy seed surpassed the diseased seed by 26 per cent in the number of seeds germinating in the laboratory, it surpassed by 70 per cent in the number of plants emerging under field conditions. The difference represents failure of viable seeds to produce plants because of the disease.

In these tests with Diplodia-infected seed, the disease resulted in the death of so many of the young sprouts before emergence as to cause a decided impairment of field stand when the seed was planted at the normal rate for this region.

If it were necessary, as has not been found to be the case in Nebraska, for a corn grower to plant seed so heavily infected with Diplodia as decidedly to curtail the stand, seed disinfection should be beneficial. Under such circumstances it would also probably be advantageous to plant the seed at a sufficiently heavy rate above normal to compensate for the reduction in field germination. Irregularity in the distribution of plants would be greater than in equal stands from healthy seed, but this has been shown by Kiesselbach, Anderson, and Lyness (*5*) to be unimportant as a yield factor. Exaggerated results of acre-yield depression are likely to follow from experimental tests planted at less than accepted standard rates. For example, as shown in the paper just cited, in a 12-year test of various planting rates, with farm-selected seed, a drop in stand from three plants to two plants per hill in checked corn resulted in a yield reduction of 7 per cent, whereas a drop from two plants to one plant per hill lowered the yield 19 per cent below that of two plants.

As indicated in these tests with farm-selected seed supplied by 18 growers, so-called planter-box seed responded to treatment with Semesan Jr. by a 2 per cent average increased field stand and one-half bushel lower acre yield. As a straight average for five years, farm-selected seed was unaffected in field stand and yielded one-half bushel less grain per acre when treated. At the North Platte substation, Zook and Jodon (*7*) obtained 1 bushel reduction in grain yield per acre on an average for seven organic mercury treatments applied to ordinary farm-selected seed. These are evidences that farm-selected seed is commonly not sufficiently infected with seedling-blight organisms to respond materially to treatment when planted at the prevailing standard rate under Nebraska conditions.

It appears evident from the statistical analysis of the plants from Diplodia-infected seed that the disease curtails the development of some of the surviving plants to such an extent as to reduce the mean plant height during growth approximately 2 or 3 inches and to increase somewhat the variability of the growing population. At

maturity, however, the relative differences have largely disappeared. Some of the affected plants may have been retarded early as a result of the disease but later outgrew the handicap. The tests supply further evidence that the disease is not systemic, as there is no greater percentage of diseased mature ears from infected than from healthy seed.

The statistical study of individual plants grown from diseased and healthy seed under conditions of essentially comparable stand suggest that the disease may have caused a doubtful reduction of 1 per cent in mean grain yield and a slight increase in the variability of individual production.

Under the conditions of these tests, stand reductions and resultant effects on yield are the only important consequences of seed infection with Diplodia. It is believed that in Nebraska the possible slight loss of viability due to this organism is negligible in its effects in the case of ordinary farm-selected seed planted at the customary rate per acre. The chief losses in viability are due to freezing injury, which is in no way related to infection with seed-borne disease organisms.

CONCLUSIONS

The most pronounced effect of planting seed corn infected with the seedling-blight organism, *Diplodia zeae* (Schw.) Lév., is a reduction in field stand. Of the viable infected seed a high percentage may die as a result of the disease, when planted, before emergence above the soil surface. While the surviving plants are on an average slightly subnormal during their growth, they tend largely to overcome this handicap by maturity. Some of the plants which are most severely attacked by the disease during their early development remain decidedly inferior and less productive. The occurrence of inferior plants increases the variability of the entire population.

The reduced stands from Diplodia-infected seed result in a greater individual yield of grain per plant under Nebraska conditions due to lessened competition. For the same reason, the occurrence of inferior plants as a result of the seedling blight is partially compensated for by the increased production of neighboring plants. The degree to which compensation for such stand deficiencies may take place will depend considerably upon their extent and upon the climatic conditions of the season. The loss in grain yield per acre may ordinarily be expected to be in inverse though not proportional relation to planting rate up to the point of providing an optimum stand for the season. In these tests there was no significant difference between the acre yields from diseased and healthy seed when the stand from the latter was adjusted to correspond with the former in number and distribution of plants.

Treatment of viable Diplodia-infected seed with a suitable disinfectant may be expected to increase the resultant field stand materially. The variability of individual plant performance will thereby also be reduced. In case of deficient stand for maximum production such disinfection should also increase the yield of grain per acre. The mean grain yield per plant may actually be reduced because of more intensive competition due to closer spacing.

Seed corn selected by ordinary farm methods and secured directly from the growers has not responded significantly, on an average, to seed treatment when planted under field conditions.

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THE ORDER, RATE, AND REGULARITY OF BLOOMING IN THE COTTON PLANT[1]

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THE WORK OF OTHER INVESTIGATORS

Many studies have been made on the flowering of cotton. Most of these have been concerned with a certain number of plants or a given length of row (*1, 7, 8, 15, 16, 17, 18*).[2] Curves have been drawn showing fluctuations in the beginning of flowering and in the daily rate due to differences in varieties, culture, or fertilizer treatment. These have taken no cognizance of bud shedding, which might greatly affect the result, nor of the rate of blooming of individual plants, but only of totals, which, in the final analysis, have hinged mainly on vigor of growth. A few studies on individual plants have been reported (*1, 4, 5, 10, 13*).

Balls (*5*) depicted the order of blooming on an individual plant, showing that this order was in the form of a spiral. He took data on the growth of the main stem and found that the variations in growth were caused, in part at least, by the influence of night temperatures, and that the variations in the growth of the main stem were reflected in the variations in the number of flowers 28 to 29 days later. The correlation between growth and night temperatures (excluding periods when the maximum was above 36° C.) was $+0.7843 \pm 0.0459$. Since Balls notes the connection between early growth and blooming, some data on intervening phenomena—intervals between appearance of fruiting branches, appearance of squares, and the mean square period—are reviewed. The correlation between the intervals in all of these and in blooming is apparently high, though not perfect, as Balls would appear to indicate.

A slowing of the growth rate Balls attributes to "senescence" or to self poisoning, a phenomenon akin to "heat poisoning," which checks the growth during periods of extremely high temperature. He maintains that flower buds have a uniform rate of development but irregularities occur as a result of variations in night temperatures.

Hammond (*9*), however, found that the square period for buds appearing in May averaged 25 days, for those appearing in June 24 days, for those in July 24 days, and for those in August 25 days.

Loomis (*11*), in a study on length of squaring period in Acala and Pima cotton, found a variation from 32.4 to 34.5 days in 1924 and 32.08 to 35.06 in 1925 in Pima, and from 27.68 to 28.6 and 28.75 to 30.42 in Acala in the same years.

McClelland (*12*), working with plants of Cleveland Big Boll cotton growing in soil of constant moisture content, found a decided tendency toward regularity in blooming, the vertical intervals being approximately three days and the horizontal intervals approximately six days. This doubling of the interval indicated tendency for a bud at

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[2] Reference is made by number (italic) to Literature Cited, p. 763.

any given location (outside a first node) to open at the same time as the bud on the second branch above but one node nearer the central stem. The order on one of the plants used for illustration is reproduced in Figure 1.

Harland (*10*) in work on sea-island cotton, found the intervals between blooms to be much like those found by McClelland on Cleveland Big Boll. The vertical intervals, as found by Harland, increase in the later growth of the plant. From the study of his plants Harland constructed a table showing the order or intervals of blooming. This table is summarized in Table 1. The reduction in time in the vertical order with the outlying nodes is due to the fact that the outer blooms on the third, fourth, fifth, and sixth nodes occurred in

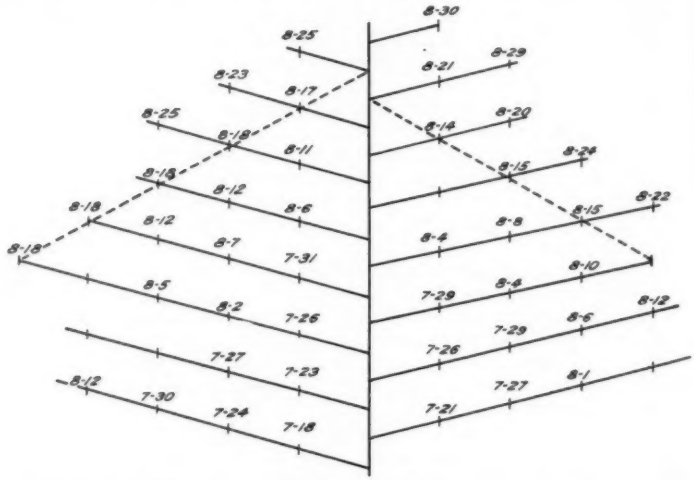

FIGURE 1.—Dates of blooming on a cotton plant, illustrating tendency toward regularity. (After McClelland)

increasing proportions only on the lower part of the plant and represent the earlier and more vigorous growth of the plant. Reference to Harland's work showing the early vertical interval on branches 1 to 20 to be 2.2 days, bears out this statement. There were no blooms at the sixth node after 51 days, at the fifth node after 65 days, at the fourth node after 78 days, nor at the third node after 81 days, counting from the appearance of the first bloom; yet the blooming period is represented as 129–137 days.

TABLE 1.—*Intervals between flowers on a typical or composite sea-island cotton plant*

[Calculated from Harland's Table X; the average plant of the manurial series]

Item	Intervals in days for node—					
	1	2	3	4	5	6
Vertical order [a]	3.2	3.3	2.7	2.6	2.3	2.2
Horizontal order (1–2, 2–3, and so on)	6.0	5.5	7.2	6.1	7.0	

[a] Harland gives intervals in vertical order between blooms at first nodes, second nodes, and so on. (Author's explanation.)

Ballard and Simpson (*3*) give some data on growth of cotton planted on different dates in Texas and South Carolina and show that the rate is speeded up by an increase in temperature.

Bailey and Trought (*2*), working with Sakel cotton in Egypt, found great regularity in blooming. If successive branches be lettered and successive nodes be numbered they show that [in theory]—

$$\left.\begin{array}{l} A^1-A^2=A^2-A^3=A^3-A^4 \\ B^1-B^2=B^2-B^3=B^3-B^4 \\ \qquad\text{etc.} \end{array}\right\} \text{and also that} \left.\begin{array}{l} A^1-B^1=A^2-B^2=A^3-B^3 \\ B^1-C^1=B^2-C^2=B^3-C^3 \end{array}\right.$$

This would be 100 per cent regular. Their results, however, showed some variation. They call attention to: (1) The remarkable constancy in blooming in individual plants; (2) the close approximation to the average for all plants; (3) the tendency to increased intervals on first nodes [vertical order] toward the top of the plant; (4) a like tendency to increased intervals between successive flowers [horizontal order] toward the end of each sympodium or fruiting branch; (5) the rhythmic nature of the flowering curves—high points occurring at intervals of 6.5 days; (6) the absence of correlation between intervals of blooming and vigor, length or thickness of sympodia, height of plant, or other characters or factors, including temperature; (7) the fact that bud shedding is a prime factor influencing flower curves made from blooms of a series of plants; and (8) that the period of square development is about 42 days rather than 28. (The American work on Egyptian cotton shows a square period of 30-35 days.)

They conclude that—

> differences of intervals are fundamental, possibly genetic, and that the regular interval is undisturbed by rate or extent of elongation of the internode, by variations in temperature during time that differentiation of the bud primordia was taking place.

Their explanation of constancy is that all internodes contain equal numbers of cells, and that an increase in the interval of blooming in later stages of growth is due to an increase in the time required for the laying down of these cells. They offer no explanation, however, of daily variations.

Martin, Ballard, and Simpson (*14*) presented results of studies made on several varieties of cotton in 1921 and 1922 at Sacaton, Ariz., Greenville, Tex., and Charleston, S. C. Probably the intervals of appearance of successive fruiting branches bear a close relation to the "vertical order of blooming at first nodes" as found in the present paper—closer one would expect than to the vertical order at the fourth node or any outlying nodes.

Beckett (*6*) determined the length of the squaring period on different types of cotton and the intervals between horizontal and vertical squares. He found the mean square period for Indian, American, and Egyptian cotton to be three to four days shorter than Loomis had found for Acala. The figures for the mean square period for Egyptian cotton in all American studies vary from 30 to 35 days, and are intermediate between the 28 days of Balls and the 42 days of Bailey and Trought.

McNamara, Hubbard, and Beckett (*13*) summarize the blooming records of 25 Lone Star cotton plants. They recorded a horizontal interval of 6.2 and a vertical interval of 2.4 days. According to their

observations the mean square period varied from 25 to 28 days both in 1923 and in 1924. They claim that rapidity in blooming "is more closely associated with vigor of plants as affected by environmental conditions than with the advance in season."

EXPERIMENTAL MATERIAL AND METHODS

The work herein reported includes data from 24 plants grown at the main station, Fayetteville, Ark., in 1923, data from 240 plants grown at the same place in 1929, and data from 124 plants grown at the cotton branch station, Marianna, Ark., in 1929. The elevation of Fayetteville is about 1,450 feet and that of Marianna is about 200 feet; the latitude of Fayetteville is 36° 0′ 14″ and that of Marianna is 34° 0′ 45″ N. Several varieties were included in the experiments to get a fair representation of both short-staple and long-staple upland cotton. The cotton was spaced at 18 inches in 42-inch rows so as to give room for a fair degree of development and a goodly number of bolls per plant. However, at Fayetteville the drought was such that normal growth was not obtained or any considerable number of flowers per plant. The range of flowers per plant was from 4 to 30, with a mean of 14.9. At Marianna a better growth was obtained, the range of flowers per plant being 8 to 60, with a mean of 30.4.

EXPERIMENTS AT FAYETTEVILLE, 1923

Table 2 gives the results obtained at Fayetteville for eight plants each of three varieties in 1923. The distribution of the blooms and the means at different locations on the plants are included.

The horizontal intervals for Trice lie between 5.36 and 6.25; for Express, 5.33 and 6.20; and for Truitt, 5.82 and 6.20, showing the slower rate of blooming of the latter variety especially.

The range of intervals in the vertical order were for Trice, between 2.43 and 3.00; for Express, 1.45 and 2.47; and for Truitt, 2.00 and 2.52, the minimum for Express (1.45) being an inexplicably wide variation from all the other results obtained. To show better these varietal variations the data are rearranged in summary form in Table 3.

TABLE 2.—*Frequency distribution of cotton bloom intervals in three varieties of cotton, Fayetteville, Ark., 1923*

HORIZONTAL ORDER

Item	Variety	Frequencies by intervals in days							Total	Mean interval and error
		3	4	5	6	7	8	9		
										Days
First and second node (A^1–A^2, B^1–B^2, etc.).	Trice		4	12	13	9	4		42	5.93±0.101
	Express	1	4	15	11	6	3	1	41	5.73± .132
	Truitt		5	10	12	5	6	1	39	6.00± .143
Second and third node (A^2–A^3, B^2–B^3, etc.).	Trice		7	19	14	3	1		44	5.36± .092
	Express	1	5	10	12	5	3		36	5.67± .135
	Truitt	1	0	10	6	2	2	1	22	5.82± .187
Third and fourth node (A^3–A^4, B^3–B^4, etc.).	Trice		5	7	7	5	1		25	5.60± .153
	Express			6	9	1	3	1	20	6.20± .176
	Truitt			1	3	0	1		5	6.20± .312
Fourth and fifth node (A^4–A^5, B^4–B^5, etc.).	Trice			2	5	5			12	6.25± .135
	Express	1	0	0	1	1			3	5.33±1.200
	Truitt				1				1	6.00

TABLE 2.—*Frequency distribution of cotton bloom intervals in three varieties of cotton, Fayetteville, Ark., 1923*—Continued

VERTICAL ORDER

Item	Variety	Frequencies by intervals in days							Total	Mean interval and error
		0	1	2	3	4	5	6		
At first node (A^1–B^1–C^1–D^1, etc.)	Trice	----	3	23	19	6	----	----	51	2.55±0.073
	Express	----	7	22	22	3	1	----	55	2.44± .077
	Truitt	----	4	26	11	4	1	----	46	2.39± .084
At second node (A^2–B^2–C^2–D^2, etc.)	Trice	1	7	17	16	5	1	----	47	2.43± .099
	Express	----	7	17	12	6	1	----	43	2.47± .102
	Truitt	2	7	12	13	6	0	2	42	2.52± .137
At third node (A^3–B^3–C^3–D^3, etc.)	Trice	1	4	12	12	3	1	1	34	2.56± .194
	Express	2	7	9	8	7	1	----	34	2.41± .146
	Truitt	2	2	2	2	3	0	1	12	2.50± .342
At fourth node (A^4–B^4–C^4–D^4, etc.)	Trice	----	6	7	5	4	2	----	24	2.54± .173
	Express	2	5	1	3	----	----	----	11	1.45± .219
	Truitt	----	----	1	----	----	----	----	1	2.00
At fifth node	Trice	----	1	----	1	----	1	----	3	3.00± .636
Average horizontal interval, all plants all branches.		----	----	----	----	----	----	----	290	5.79± .047
Average vertical interval, all plants all nodes.		----	----	----	----	----	----	----	403	2.48± .037

TABLE 3.—*Summary by varieties showing intervals of blooming in the cotton plant, Fayetteville, Ark., 1923*

Variety	Horizontal intervals				Vertical intervals			
	Location on plants	Plants	Counts	Mean interval and P. E.	Location on plants	Counts	Mean interval and P. E.
		Number	*Number*	*Days*		*Number*	*Days*
Trice	First to second nodes	8	42	5.93±0.101	First node	51	2.55±0.073
	Second to third nodes	8	44	5.36± .092	Second node	47	2.43± .099
	Third to fourth nodes	8	25	5.60± .153	Third node	34	2.56± .194
	Fourth to fifth nodes	8	12	6.25± .135	Fourth node	24	2.54± .173
					Fifth node	3	3.00± .636
Express	First to second nodes	8	41	5.73± .132	First node	55	2.44± .077
	Second to third nodes	8	36	5.67± .135	Second node	43	2.47± .102
	Third to fourth nodes	8	20	6.20± .176	Third node	34	2.41± .146
	Fourth to fifth nodes	8	3	5.00± .840	Fourth node	11	1.45± .219
Truitt	First to second nodes	8	39	6.00± .143	First node	46	2.39± .084
	Second to third nodes	8	22	5.82± .187	Second node	42	2.52± .137
	Third to fourth nodes	8	5	6.20± .312	Third node	12	2.50± .342
	Fourth to fifth nodes	8	1	6.00	Fourth node	1	2.00

EXPERIMENTS AT FAYETTEVILLE, 1929

In 1929 counts were made on 240 plants of five varieties at Fayetteville. The season was very dry and growth was very unsatisfactory. Data were recorded between the dates July 28 and August 24, though blooming had practically ceased by August 16.

The number of blooms per plant ranged from 4 to 32, with a mean of approximately 15 per plant. As the plants were spaced at 18 inches in 42-inch rows, this average is quite low.

Table 4 gives a summary of the results obtained in both horizontal and vertical order with the several varieties, averages for the horizontal and vertical intervals for each variety, and for all varieties combined. Ratios between horizontal and vertical intervals are also shown. The longest intervals between blooms on fruiting branches were found on the Rowden and Express varieties. Triumph had a shorter interval than Trice, which has been reputed to be a rapid-

blooming variety. When the intervals in a vertical direction are considered, Delfos is found to be the most rapid bloomer and Triumph the slowest, the rate varying from 2.30 to 2.54 days, with an average for all varieties of 2.36 days. The intervals on different portions of the plants likewise varied widely (Table 5). For both years studied an increase in the interval is found toward the distal ends of the fruiting branches. In 1923 the increase was from 5.885 to 6.0, though the gain was not consistent. In 1929, when the number of plants was larger, a consistent increase from 5.898 to 6.226 was found.

TABLE 4.—*Summary by varieties of data on rate and regularity of blooming in different varieties of the cotton plant, Fayetteville, Ark., 1929*

Variety and and number of plants	Horizontal intervals			Vertical intervals			Ratio of intervals X:1
	Location on plants	Counts	Mean interval and P. E.	Location on plants	Counts	Mean interval and P. E.	
		Number	*Days*		*Number*	*Days*	
Rowden, 40 plants	First to second nodes	133	6.06±0.063	First node	185	2.26±0.058	2.68
	Second to third nodes	55	6.40± .120	Second node	105	2.54± .096	2.52
	Third to fourth nodes	14	7.43± .223	Third node	27	2.07± .222	3.59
Delfos, 40 plants	First to second nodes	163	5.52± .050	First node	213	2.35± .042	2.35
	Second to third nodes	133	5.57± .061	Second node	175	2.22± .055	2.51
	Third to fourth nodes	53	6.19± .097	Third node	125	2.21± .084	2.80
	Fourth to fifth nodes	9	6.33± .237	Fourth node	29	2.83± .213	2.23
	Fifth to sixth nodes			Fifth node	4	2.75± .279	
Trice, 50 plants	First to second nodes	222	5.83± .057	First node	289	2.48± .043	2.35
	Second to third nodes	132	5.98± .069	Second node	191	2.41± .058	2.48
	Third to fourth nodes	50	5.98± .148	Third node	94	2.17± .094	2.75
	Fourth to fifth nodes	12	6.67± .380	Fourth node	28	1.89± .208	3.53
	Fifth to sixth nodes	1	9.00	Fifth node	5	1.00± .381	9.00
Express, 50 plants	First to second nodes	155	6.34± .060	First node	238	2.46± .054	2.58
	Second to third nodes	47	6.94± .098	Second node	117	2.28± .083	3.04
	Third to fourth nodes	3	6.33± .184	Third node	20	1.18± .177	4.36
				Fourth node	2	2.50± .239	
Triumph, 60 plants	First to second nodes	51	5.61± .140	First node	100	2.61± .074	2.15
	Second to third nodes	56	5.48± .143	Second node	76	2.42± .087	1.31
	Third to fourth nodes	31	5.55± .199	Third node	62	2.39± .012	2.32
	Fourth to fifth nodes	10	5.60± .218	Fourth node	12	3.00± .424	1.87
	Fifth to sixth nodes	1	6.00	Fifth node	3	4.33± .662	
Average for:							
Rowden		202	6.24		317	2.34	2.66
Delfos		358	5.66		546	2.30	2.46
Trice		417	5.93		607	2.37	2.50
Express		205	6.48		377	2.34	2.77
Triumph		149	5.55		253	2.54	2.18
Total or average		1,331	5.947		2,100	2.362	2.52

TABLE 5.—*Summary by location on plants of results on regularity of blooming of cotton at Fayetteville, Ark., 1923 and 1929*

Year and variety	Intervals of blooming between given nodes, horizontal order				Intervals of blooming between successive branches at respective nodes, vertical order				
	1-2	2-3	3-4	4-5	1	2	3	4	5
1923:	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Trice	5.93	5.36	5.60	6.25	2.55	2.43	2.56	2.54	3.00
Express	5.73	5.67	6.20	5.00	2.44	2.47	2.41	1.45	
Truitt	6.00	5.82	6.20	6.00	2.39	2.52	2.50	2.00	
Average	5.89	5.57	5.92	6.00	2.46	2.43	2.49	2.19	3.00
Total counts	122	102	50	16	152	132	80	36	3
1929:									
Rowden	6.06	6.40	7.43		2.26	2.54	2.07		
Delfos	5.52	5.57	6.19	6.33	2.35	2.22	2.21	2.83	2.75
Trice	5.83	5.98	5.98	6.67	2.48	2.41	2.17	1.89	1.00
Express	6.34	6.94	6.33		2.46	2.28	1.18	2.50	
Triumph	5.61	5.48	5.55	5.60	2.61	2.42	2.39	3.00	4.33
Average	5.90	5.95	6.11	6.23	2.42	2.36	2.16	2.48	2.42
Total counts	724	423	151	31	1,025	664	328	69	12

In considering the vertical intervals at different distances from the main stems, less variation is noted than in the horizontal intervals, and some of these the writers are unable to explain. In 1923 the vertical interval at the fourth node out from the main stem was somewhat smaller than the others, and this was unduly increased (due to smallness of numbers mainly) at the fifth node; in 1929 the small interval was found at the third node. This increase in horizontal intervals agrees with the averages as calculated from Harland (Table 1), but there is disagreement in the holding constant of the vertical intervals. Harland's results showed consistent decrease, but perhaps this disagreement may be explained by the fact that he had a longer blooming period (129 to 137 days) and larger plants having 20 to 40 fruiting branches and as high as 15 blooms per fruiting branch and 175 (or more) potential blooms per plant.

THE EFFECT OF SEASON

Since data are available on two varieties, Trice and Express, covering two different seasons, the influence of season on intervals, if any, should be apparent. The monthly rainfall and the average maximum, minimum, and monthly temperatures for the summer months of 1923 and 1929 are given in Table 6.

TABLE 6.—*Rainfall and temperature records for June, July, and August, 1923 and 1929, in northwest Arkansas*

Item	June	July	August
1923—Rainfall, inches	5.20	3.53	2.43
Average maximum temperatures, °F	84.5	90.7	95.8
Average minimum temperatures, °F	65.0	67.3	70.1
Average monthly temperatures, °F	76.7	79.9	81.9
1929—Rainfall, inches	3.68	3.07	1.29
Average maximum temperatures,[a] °F	82.7	94.4	95.9
Average minimum temperatures,[a] °F	63.2	72.4	67.1
Average monthly temperatures, °F	76.2	81.2	80.8

[a] For Eureka Springs; no data for Fayetteville.

A record of the blooming on Trice was begun on July 23 in 1923 and on July 28 in 1929. On Express the dates were July 26 and July 29, respectively. There were a few straggling blooms prior to these dates. Table 7 gives the mean interval measurements obtained. On both varieties there is a tendency toward a slight lengthening of the horizontal intervals and a shortening of the vertical intervals in 1929 as compared to the results obtained in 1923. More data will be needed, however, before a definite conclusion can be reached as to the effect of season on the intervals of blooming, though the increase in the horizontal intervals, especially with Express, is apparently significant.

TABLE 7.—*Seasonal variation in the intervals of blooming of Trice and Express cotton plants, Fayetteville, Ark., 1923 and 1927*

Variety and blooming measures	Location on plants	Counts and intervals in—				Gain or loss in intervals
		1923 (8 plants)		1929 (50 plants)		
		Counts	Mean interval and P. E.	Counts	Mean interval and P. E.	
Trice:		*Number*	*Days*	*Number*	*Days*	
Horizontal	First to second nodes	42	5.93±0.101	222	5.83±0.057	−0.10±0.118
	Second to third nodes	44	5.36± .092	132	5.98± .069	+.62± .115
	Third to fourth nodes	25	5.60± .153	50	5.98± .148	+.38± .214
	Fourth to fifth nodes	12	6.25± .135	12	6.67± .380	+.42± .403
Vertical	First node	51	2.55± .073	289	2.48± .043	−.07± .085
	Second node	47	2.43± .099	191	2.41± .058	−.02± .115
	Third node	34	2.56± .194	94	2.17± .094	−.39± .215
	Fourth node	24	2.54± .173	28	1.89± .208	−.65± .270
	Fifth node	3	3.00± .636	5	1.00± .381	−2.00± .741
Express:						
Horizontal	First to second nodes	41	5.73± .132	155	6.34± .060	+.61± .145
	Second to third nodes	36	5.67± .135	47	6.94± .098	+1.27± .167
	Third to fourth nodes	20	6.20± .176	3	6.33± .184	+.13± .254
	Fourth to fifth nodes	3	5.00± .840			
Vertical	First node	55	2.44± .077	238	2.46± .054	+.02± .094
	Second node	43	2.47± .102	117	2.28± .083	−.19± .131
	Third node	34	2.41± .146	20	1.18± .177	−1.23± .229
	Fourth node	11	1.45± .219	2	2.50± .239	+1.05± .324
Average, Trice:						
Horizontal		123	5.690± .064	416	5.92± .042	+.230± .076
Vertical		159	2.524± .053	607	2.37± .033	−.154± .062
Average, Express:						
Horizontal		100	5.780± .088	205	6.477± .052	+.697± .102
Vertical		143	2.366± .056	377	2.336± .044	−.030± .071

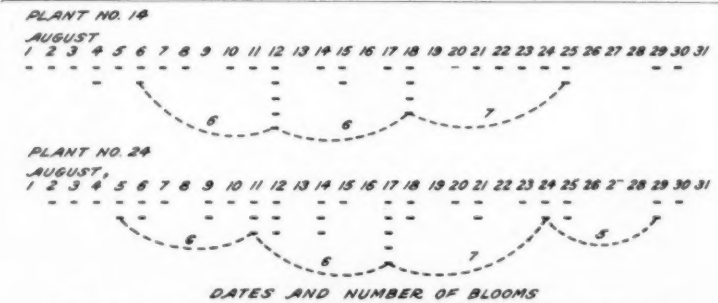

FIGURE 2.—Frequency of blooms in August of two cotton plants showing cyclic tendencies

THE RHYTHMIC CYCLE OF COTTON BLOOMING

Bailey and Trought (*2*) called attention to the fact that flowering curves are rhythmic, high points occurring at intervals of 6.5 days. The explanation of the regular appearance of peaks in the curves lies in the regularity of blooming in the individual plants. In theory, if the vertical interval is 3 and the horizontal interval 6, and nothing disturbs the regularity, blooms should occur on any one plant only at 3-day intervals, and there would be no true daily curve for individual plants. If the regularity is upset (from whatever cause) and if the intervals are not exactly three or six days, nor yet in an exact ratio of 1:2, there are many blooms on intermediate days and the charted record becomes a curve, with peaks at intervals. Bud shedding also tends to interrupt the expected result. The fact that different plants begin blooming on different dates is, however, the main reason for a smoothing of these curves. (Fig. 2.)

If a majority of the plants began blooming on the same day, the tendency toward regularity would be great enough to account for peaks in the curves at regular intervals, though this does not explain why the high points occur near the 6-day interval rather than on the three.

A study of the flowering data from certain plants will illustrate the idea. Figure 2 gives the number of flowers occurring on the different days during August on the two plants used for illustration in the article by McClelland (*12*) to which reference has been made. The rhythmic period, as shown in Figure 2, varies from five to seven days, but probably averages very close to the mean length of the horizontal intervals as found at any given place or season on any given variety of cotton. A slight tendency toward a 3-day cycle is noted only in plant No. 24. On smaller plants, such as were used in 1929, a study

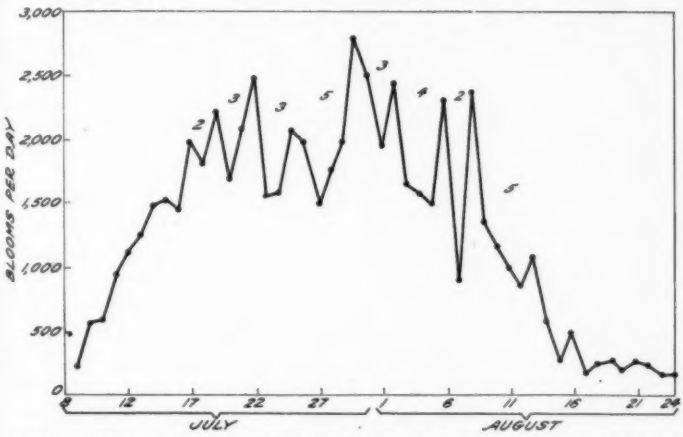

FIGURE 3.—Flowering curves of 30 check rows of Rowden cotton, Scott, Ark., July 8 to August 24, 1929

of this variation in blooming can hardly be made. The tendency toward a smoothing of the flowering curve with any larger number of plants is readily explained. If conditions are uniform and such that most of the plants begin blooming on the same day, and if they preserve the same degree of regularity, as it seems they should, then peaks in the curves at more or less regular intervals are bound to occur. Inspection of the data from 30 rows, 44 inches by 132 feet, of Rowden cotton in 1929 (fig. 3) reveals a great irregularity in the number of blooms per day but no cyclic tendency. With the 240 plants used at Fayetteville in 1929 the peaks occurred at an average of four days with one 3-day and one 5-day interval alternating with those of four days. (Fig. 4.)

RELATION OF VIGOR OF PLANT TO LENGTH OF BLOOMING INTERVAL

It was thought that the very unfavorable conditions at Fayetteville in 1929 might have influenced the intervals of blooming and that this influence would be most apparent in the plants of least vigor; in other words, that the intervals would be smaller in plants having

the larger number of flowers. To determine this point the average horizontal and vertical intervals for each of the 240 plants were computed. These were then grouped in classes with a class interval of 0.2. The range horizontally was from 4.7 to 7.5 days and vertically from 1.3 to 3.7 days, the few extreme variations being included in the outer groups.

The coefficients of correlation were: Between number of flowers per plant and average horizontal interval per plant, $r = -0.132 \pm 0.0425$; between number of flowers per plant and average vertical interval per plant, $r = -0.251 \pm 0.0415$.

These correlations appear to be significant though very small, indicating that vigor of growth as shown by number of flowers per

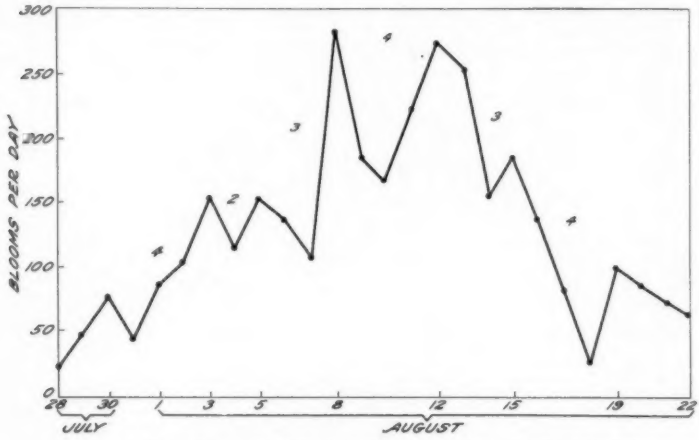

FIGURE 4.—Flowering curve of 240 cotton plants grown at Fayetteville, Ark., 1929

plant exercised a small influence in reducing the length of the blooming intervals.

EXPERIMENTS AT MARIANNA, 1929

In cooperation with the cotton branch station at Marianna records were kept on 124 plants in 1929. Plantings of Acala, Trice, and Express were made on May 6 and May 21, and one planting of Delfos was made on May 6. The Trice, Delfos, and Express varieties are common to the two tests of 1929.

Table 8 summarizes the results of the findings at the cotton branch station. With all varieties, the intervals increased horizontally to the interval between the third and fourth nodes, after which irregularity occurred. In the vertical order, it was generally found that there was little difference in intervals at first or second nodes, there was usually some shortening of intervals at the third nodes, and beyond this the results were more variable.

TABLE 8.—*Summary by varieties showing rate and regularity of blooming in cotton plants, cotton branch station, Marianna, Ark., 1929*

Variety, number of plants, and planting date	Horizontal intervals			Vertical intervals			Ratio of intervals X:1
	Location on plants	Counts	Mean interval and P. E.	Location on plants	Counts	Mean interval and P. E.	
		Number	*Days*		*Number*	*Days*	
Acala, 19 plants, May 6.	First to second nodes	141	6.18±0.079	First node	156	2.75±0.075	2.24
	Second to third nodes	111	6.44±.085	Second node	119	2.65±.087	2.43
	Third to fourth nodes	63	6.75±.129	Third node	86	2.50±.118	2.70
	Fourth to fifth nodes	22	6.91±.208	Fourth node	38	2.29±.211	3.01
	Fifth to sixth nodes	8	6.50±.337	Fifth node	9	2.78±.459	2.33
Acala, 18 plants, May 21.	First to second nodes	117	5.95±.074	First node	138	2.39±.061	2.49
	Second to third nodes	78	6.26±.099	Second node	88	2.42±.103	2.58
	Third to fourth nodes	33	6.85±.141	Third node	50	2.28±.177	3.00
	Fourth to fifth nodes	5	6.00±.060	Fourth node	15	1.80±.231	3.33
				Fifth node	2	1.50±.239	
Trice, 20 plants, May 6.	First to second nodes	189	5.62±.066	First node	195	2.47±.056	2.27
	Second to third nodes	149	5.68±.078	Second node	157	2.43±.074	2.33
	Third to fourth nodes	93	5.86±.110	Third node	106	1.95±.102	3.00
	Fourth to fifth nodes	45	5.82±.151	Fourth node	64	2.55±.161	2.28
	Fifth to sixth nodes	21	6.00±.187	Fifth node	20	2.70±.262	2.22
	Sixth to seventh nodes	3	5.00±.318	Sixth node	6	1.83±.604	2.73
Trice, 20 plants, May 21.	First to second nodes	130	5.53±.077	First node	152	2.28±.060	2.42
	Second to third nodes	76	5.86±.104	Second node	87	2.33±.094	2.51
	Third to fourth nodes	32	6.34±.180	Third node	46	2.19±.163	2.89
	Fourth to fifth nodes	12	5.83±.236	Fourth node	18	2.45±.236	2.38
	Fifth to sixth nodes	4	5.50±.377	Fifth node	9	1.14±1.146	4.82
Express, 19 plants, May 6.	First to second nodes	148	5.93±.077	First node	164	2.49±.059	2.38
	Second to third nodes	86	6.48±.104	Second node	123	2.54±.087	2.55
	Third to fourth nodes	42	6.67±.165	Third node	65	2.35±.135	2.84
	Fourth to fifth nodes	12	6.25±.357	Fourth node	29	2.69±.237	2.32
	Fifth to sixth nodes	2	3.50±.238	Fifth node	6	1.60±.451	2.19
Express, 9 plants, May 21.	First to second nodes	33	5.82±.162	First node	53	2.57±.125	2.26
	Second to third nodes	14	6.00±.361	Second node	18	2.89±.175	2.07
	Third to fourth nodes	6	6.50±.545	Third node	7	2.71±.589	2.40
				Fourth node	3	1.67±1.051	
Delfos, 19 plants, May 6.	First to second nodes	164	5.24±.075	First node	175	2.54±.059	2.06
	Second to third nodes	126	5.50±.086	Second node	131	2.38±.088	2.31
	Third to fourth nodes	80	5.73±.107	Third node	101	2.56±.107	2.23
	Fourth to fifth nodes	45	5.53±.157	Fourth node	51	2.59±.174	2.13
	Fifth to sixth nodes	9	6.33±.106	Fifth node	24	2.00±.242	3.16
Average:							
Acala, early		345	6.42		408	2.63	2.44
Acala, late		233	6.18		293	2.34	2.64
Trice, early		500	5.71		548	2.37	2.41
Trice, late		254	5.74		312	2.25	2.53
Express, early		290	6.20		387	2.48	2.50
Express, late		53	5.94		81	2.62	2.27
Delfos, early		424	5.46		482	2.48	2.20
Total or average		2,099	5.90		2,511	2.44	2.41

Trice is shown to be a faster bloomer than either Acala or Express. Delfos also had shorter horizontal intervals, but was only average in the length of the vertical intervals.

It is evident that there was reduction in horizontal intervals caused by later planting; in vertical intervals, two of the three varieties showed this reduction. In other words, the rate of blooming was accelerated by delaying the planting until the arrival of longer days and warmer weather.

THE EFFECT OF ALTITUDE ON BLOOMING INTERVAL

By comparing the data taken at Fayetteville with those from Marianna the effect of different environmental conditions may be observed. The comparison shown in Table 9 indicates a lengthening of the blooming intervals, horizontally, due to greater altitude and lower temperatures. On the other hand, the vertical intervals are shortened in Delfos and Express, but are slightly lengthened in Trice at the higher altitude.

TABLE 9.—*Effect of altitude on blooming intervals of three varieties of cotton*

Variety	Average horizontal intervals at—		Average vertical intervals at—	
	Fayetteville (elevation 1,450 feet)	Marianna (elevation 200 feet)	Fayetteville (elevation 1,450 feet)	Marianna (elevation 200 feet)
	Days	*Days*	*Days*	*Days*
Trice	5.93	[a] 5.72	2.37	[a] 2.32
Delfos	5.66	5.46	2.30	2.48
Express	6.48	[a] 6.16	2.34	[a] 2.50

[a] Average of the 2 plantings.

SUMMARY AND DISCUSSION

Cotton exhibits a greater or less tendency toward regularity in its blooming. The regularity is interrupted by many different causes and the rate is influenced by variety, seasonal conditions, location, cultural methods, and possibly other factors.

The intervals between the appearance of successive fruiting branches and the appearance of successive squares, as determined by other investigators, show a close similarity to the intervals between blooms in a vertical and horizontal direction as indicated in the present paper.

A cyclic tendency in blooming in grouped plants is due to the tendency toward regularity in the individual plants. That the breaks in the flowering curves are not regular and pronounced is due (1) to conditions which upset the regularity in the individual plants, (2) to the fact that these plants begin blooming on different dates, and (3) to injuries and shedding, which to some extent modify results.

The horizontal interval of blooming varies on an average less than 0.4 from a 6-day interval and consistently increases at outer nodes of fruiting branches over the intervals found between nodes nearer the central stem. In other words, the early intervals are shorter than the later ones.

The average for vertical order of blooming is in very few instances outside the range 2.3 to 2.8 days. Usually the vertical interval decreased at the outer nodes, since blooms occurred at such nodes only on the earlier or lower fruiting branches and were a product of the vigorous growth period of the plants.

The ratio between the horizontal and vertical blooming intervals, though varying greatly, was in most instances between 2.6 and 2.1. To be strictly regular this ratio, it would appear, must be exactly 2, but the vertical intervals have been unduly shortened, increasing the ratios.

A study of the data of all workers shows but slight differences in the rapidity of blooming of cotton, due to difference in species, variety, altitude, latitude, season, or various cultural practices. To make cotton "bloom fast" is almost impossible, but the number of blooms per plant or per row or per day can be increased by increasing the size of the plant. The plants that produce 75 to 100 blooms will show so-called faster rates of blooming than plants that produce 20 to 30 blooms.

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A COMPARISON OF METHODS FOR DETERMINING THE VOLUME-WEIGHT OF SOILS [1]

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INTRODUCTION

Since some of the soils of New Mexico are extremely variable in texture, structure, and stratification, they present many problems to the investigator who tries to determine their volume-weights.[2] Because of these varying conditions, several of the common methods used in making volume-weight determinations can not be depended upon to give satisfactory results. Therefore, experimental work was undertaken to determine the most suitable method for use under these conditions. The paraffin-immersion method,[3] being impractical here, was not considered in these tests. Comparisons were made between the 1-foot cylinder method, the rubber-tube method,[4] the viscous-fluid method,[5] the improved soil-tube method,[6] and a revised sand method.[7]

EQUIPMENT AND PROCEDURE

The cylinder used in the tests with the 1-foot cylinder method was made from a piece of large water pipe. (Fig. 1.) A section 13.25 inches in length was remodeled in a machine shop so that the inside diameter was 3.61 inches and the cylinder was uniform and smooth throughout.

Approximately one thirty-second inch was cut away from the outside of the cylinder from the top to within 1 inch of the lower end. From 1 inch downward the cylinder was beveled to a cutting edge, thus making it flush with the inside diameter and leaving a small flange on the outside to reduce friction. A driving plug was cut from a solid piece of material so that it was about 1.25 inches in thickness and of the same diameter as the outside of the cylinder. A seat about 0.2 inch in depth (it should have been deeper, so the plug would have remained in position better) was cut out on the lower side of the plug so that it seated snugly on top of and partially inside of

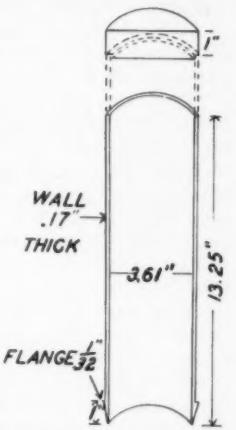

FIGURE 1.—Longitudinal section of cylinder and driving plug, with the approximate dimensions, used in the 1-foot cylinder method of soil volume-weight determination

[1] Received for publication Jan. 13, 1931; issued June, 1931.

[2] The volume-weight, or apparent specific gravity, of a soil is a figure which represents the ratio between the weight of a given volume of undisturbed water-free soil and the weight of an equal volume of water.

[3] SHAW, C. F. A METHOD FOR DETERMINING THE VOLUME WEIGHT OF SOIL IN FIELD CONDITION. Jour. Amer. Soc. Agron. 9: 38–42. 1917.

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the cylinder. Owing to the use of this plug, the cylinder was not damaged by driving with a 2-pound hammer.

The rubber tube used in these trials was 61 inches in length and slightly more than 1.5 inches in diameter. Rubber bandages, such as are commonly used by the medical profession, were made up into a tube of the desired dimensions by a local tire vulcanizer. This rubber seems to have been slightly stiff for this purpose. From a specific-gravity test on the rubber, it was found that 86 ml. of water were displaced by the tube.

The oil used with the viscous-fluid method is commonly known as "600 W." (Preliminary tests were made with a heavy road oil, but it penetrated so rapidly into the sandy strata that an accurate measurement could not be secured.) The volumes were obtained by weighing the oil used for each hole. This weight was multiplied by 1.1287 as a correction factor, which was obtained by dividing 100 by 88.6 to get the correct volume. A test on the oil showed that 100 ml. weighed 88.6 gm.

The tube used with the improved soil-tube method was like the one described by Veihmeyer. He stated that this tube was used with satisfactory results in making volume-weight determintions.

When the sand method was used the volume of the hole was determined by measuring volumetrically the sand required to fill the hole which had been previously drilled with an auger. The sand was obtained from a gravel pit and screened. Only the particles which would pass through a 2-mm. sieve and which were retained on a 1-mm. sieve were used. Tests with various sized particles showed that this size gave the most consistent results, so far as pouring and compaction were concerned. As a preliminary test an old corroded water pipe, which was sufficiently rough to resemble the wall of a hole in the soil, about 1.5 inches in diameter and 5 feet in length, was placed upright and filled with the sand, which was poured continuously from a 500-ml. graduated cylinder through a funnel with an opening of about three-eighths of an inch. The same funnel was used to fill the cylinder with sand. An effort was made to pour the sand into the cylinder in a continuous and uniform stream until it was almost full. The remaining portion was filled by hand, extreme care being used to prevent jarring, as the least blow would have caused the sand to settle. Repeated trials showed that the milliliters of sand used in filling the pipe multiplied by 0.9501 gave the correct volume, within reasonable limits, which had been previously determined with water. However, this factor was not used in making volume-weight calculations, because it was thought that the inside of the pipe was not exactly like the holes in the soil, so far as roughness was concerned. The factors used in the tests were calculated from the field work, that is, the total dry weight of the samples was divided by the average volume weight, as determined by the cylinder method, and this figure was divided by the total cubic centimeters of sand; the resulting figure was the correction factor. The number of cubic centimeters of sand for each sample was then multiplied by this factor to secure the volume weights of each sample.

The auger employed in making the holes when the rubber-tube method, the viscous-fluid method, and the sand method were used was made of a 1.5-inch wood bit welded to about 5 feet of ½-inch

water pipe with a T joint at the top and with 8-inch pieces of pipe for a handle.

The soil in which tests were made was extremely variable in structure and texture and is classed as Gila clay adobe.[8] The surface soil for a depth of 18 to 20 inches was a clay adobe. Below this was a 4-inch layer of silty fine sand; then a layer about 6 inches in thickness composed of an intermingling of sand, silt, and adobe. Next was a 1-inch layer of fine tight clay, and then about 30 inches of material varying from adobe with accumulations of sand at the top to clean medium sand at the bottom. The change in texture from the top to the bottom of this layer was gradual.

To facilitate the making of these tests a trench was dug 2.5 feet wide, 6 feet deep, and about 20 feet long. Ten groups of tests were made along one side of this trench, all of which were included in an area about 18 feet long and 2.5 feet wide. Ten tests were made with the cylinder, 20 with the soil tube, 20 with sand, 20 with oil, and 20 with the rubber tube. The holes used for sand were first used for the

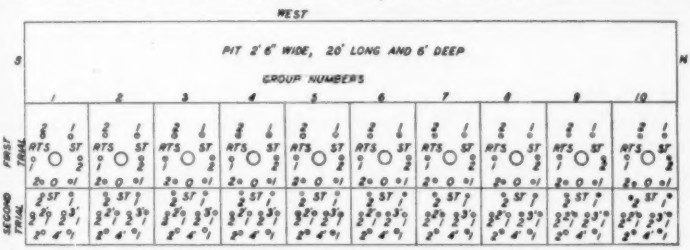

FIGURE 2.—Arrangement of pit, groups, and holes used in both trials. The sand method was used on the last two rows. See text for explanation

rubber-tube method. Figure 2 shows the arrangement of groups, holes, and pit.

The pit was dug so that as little soil as possible would be disturbed in taking the samples for the cylinder method. A large number of samples were taken in a very small area and theoretically there should have been very little difference in the soils from different groups. In the center of each group and about 18 inches from the edge of the pit a place was marked for the cylinder-method sample. On the east side of each group two holes were marked O for the oil method. On the northwest side two holes were marked ST for the soil-tube method, and on the southwest side two were marked RTS for the rubber-tube and sand methods. The holes in each group were from 4 to 6 inches apart and about 6 inches from the hole used for the cylinder method. The oil holes were placed on the far side from the pit so that the oil could be drained out after the cylinder-method sample had been taken and then used for the next set of holes. In doing this a small amount of sand accumulated in the oil, which probably changed its specific gravity and resulted in a lower volume-weight figure. However, this practice did not affect the spread of the results, since both the highest and lowest volume-weight figures were obtained from holes in which new oil was used.

The holes for the oil method, sand method, and rubber tube method were all made with the auger described above. The holes for the soil tube method were made with the improved soil tube. No

[8] NELSON, J. W., and HOLMES, L. C. SOIL SURVEY OF MESILLA VALLEY, NEW MEXICO-TEXAS. U. S. Dept. Agr., Bur. Soils Field Oper. 1912, Rpt. 14: 2011-2045, illus. 1915.

attempt was made to take any of these samples in foot sections. The soil tube was inserted in each hole from three to five times in taking the entire sample. When the cylinder method was used the samples were taken in 1-foot sections and the five weights totaled with the five volumes in making the volume-weight calculations. An attempt was made to take all samples for all methods to a depth of 60 inches, but with the cylinder method a slightly greater depth was obtained, although not enough to affect the results. The holes for the oil, soil tube, sand, and rubber-tube methods were made first, then the cylinder for the cylinder method was driven down 1 foot and excavated with a pick, chisel, saw, and shovel. It was found that very little if any compaction resulted from the use of the cylinder. Standard level marks were established with a stake about 2 feet from these holes, so that accurate measurements could be made on the depth of each sample. All of the soil taken from each hole was dried at about 105° C. and volume-weight calculations were made after the necessary correction factors had been applied. Table 1 shows the volume weights obtained by the different methods.

EXPERIMENTAL DATA

The results obtained by the cylinder method are more uniform and consistent than those obtained by the other methods and it is believed that they are accurate and reliable; therefore, they are used as the standard by which the results from the other methods are compared with it as a standard.

TABLE 1.—*Individual soil volume-weight determinations made by different methods*

[First group of tests]

Group No.	Hole No.	Soil volume weight as determined by method indicated					
		Cylinder	Sand	Improved soil tube		Rubber tube	Viscous fluid
				First trial	Second trial		
1	1	1.45	1.48	1.28	1.33	1.35	1.05
1	2		1.48	1.38	1.38	1.39	1.86
2	1	1.45	1.26	1.44	1.40	1.20	1.19
2	2		1.36	1.43	1.38	1.29	1.05
3	1	1.42	1.51	1.43	1.42	1.40	1.33
3	2		1.49	1.38	1.39	1.37	1.23
4	1	1.45	1.40	1.39	1.39	1.35	1.32
4	2		1.45	1.30	1.37	1.38	1.25
5	1	1.45	1.46	1.43	1.42	1.39	1.26
5	2		1.47	1.41	1.40	1.38	1.21
6	1	1.44	1.45	1.35	1.40	1.36	1.27
6	2		1.40	1.42	1.41	1.40	1.31
7	1	1.42	1.45	1.37	1.28	1.37	1.26
7	2		1.48	1.23	1.17	1.30	1.29
8	1	1.41	1.39	1.36	1.34	1.30	1.30
8	2		1.44	1.35	1.34	1.33	1.24
9	1	1.39	1.42	1.33	1.38	1.34	1.28
9	2		1.43	1.38	1.35	1.35	1.28
10	1	1.39	1.38	1.38	1.32	1.29	1.31
10	2		1.44	1.36	1.34	1.36	1.31
Average		1.43±0.005	1.43±0.008	1.37±0.008	1.36±0.008	1.34±0.007	1.28±0.023

These tests were located, unavoidably, at right angles to and a short distance from a row of young shade trees. In making these determinations it was noticed that the roots increased in number from the first to the last group, and it is probable that the decrease

shown in the volume weights for the cylinder method is due to this factor. However, this condition was not so noticeable where the other methods were used, probably because the smaller instruments had a tendency to spread the roots rather than cut them off. Also a little more variation might have been secured if 20 determinations had been made instead of 10.

The determinations by the sand method, because of the correction factor used, gave the same average result as the cylinder method, but more variation was in evidence. The results obtained by the improved soil-tube method, shown in the fifth and sixth columns of Table 1, are about as variable as those obtained by the sand method, but are lower than those secured both by the cylinder and the sand methods. The results from the improved soil-tube method, shown in the fifth column, were secured from the first trial. The variation is about the same as for the sand method, but the average volume weight is lower than for the cylinder method. In order to check these data a second test was made, the results of which are shown in the sixth column. The average results for the second trial were practically the same as for the first and the variation was practically the same. This method is convenient and rapid, and it is probable that were the results multiplied by 1.0476, an accurate value would be secured. The results from the rubber-tube method were about the same as for the improved soil-tube method, but lower than for the cylinder method, and showed considerably more variation. Apparently a correction factor should be used in connection with this method to secure reliable results. The viscous-fluid method (where 600 W. oil was used) gave a low average volume weight with considerable variation in the individual determinations, and for this reason can not be recommended for use under such conditions as prevailed in these experiments.

Only half as many determinations were made by the cylinder method as by the others, and it would hardly be proper to compare 10 cylinder-method tests with 20 for the other methods. It was for this reason that Table 2 was prepared. Section A of this table shows the average volume weights, standard deviation, coefficient of variation, highest volume weight, lowest volume weight, and spread for all determinations for the various methods. Section B shows the same comparisons as section A, except that a summary of data from holes numbered 1 is used. In section C a summary of data from holes numbered 2 from all except the cylinder method was used. The first column of figures in the three sections of this table is the same, as duplicate holes were not used in connection with this method. A glance at section A shows that the cylinder method gave more satisfactory results than the others and that the viscous-fluid method gave the least reliable results. In section B, where only 10 determinations for each method were considered, the cylinder method also appears reliable and satisfactory, as compared with the other methods. The viscous-fluid method appears to be the least reliable, since the average volume-weight figure is very low and the spread, standard deviation, and coefficient of variation are all higher than for the other methods. The sand method, the improved soil-tube method, and the rubber-tube method are about the same so far as standard deviation and coefficient of variation are concerned.

The sand method presents more spread in the data than either of the other two in this section. In section C the same comparisons are made. The sand method is found to present less spread than the improved soil-tube method and it also shows a very small coefficient of variation. The viscous-fluid method appears to be very unsatisfactory in this section, because of the lack of consistency in the data.

TABLE 2.—*Comparisons of results obtained in soil determinations using various volume-weight methods*

SECTION A

	Values [a] given by the method indicated					
	3.61-inch cylinder	Sand	Improved soil tube		Rubber tube	Viscous fluid
			First trial	Second trial		
Trials, number	10	20	20	20	20	20
Average volume weights	1.43±0.005	1.43±0.008	1.37±0.008	1.36±0.008	1.34±0.007	1.28±0.023
Standard deviation	.0235	.0557	.0529	.0566	.0480	.1529
Coefficient of variation, per cent	1.64	3.90	3.86	4.16	3.58	11.95
Highest volume weight	1.45	1.51	1.44	1.42	1.40	1.86
Lowest volume weight	1.39	1.26	1.23	1.17	1.20	1.05
Spread	.06	.25	.21	.25	.20	.81

SECTION B

Trials, number	10 (No. 1)	10 (No. 1)	10 (No. 1)	10 (No. 1)	10 (No. 1)	10 (No. 1)
Average volume weights	1.43±0.005	1.42±0.010	1.38±0.010	1.37±0.010	1.34±0.012	1.26±0.017
Standard deviation	.0235	.0469	.0480	.0447	.0557	.0787
Coefficient of variation, per cent	1.64	3.30	3.48	3.26	4.16	6.25
Highest volume weight	1.45	1.51	1.44	1.42	1.40	1.33
Lowest volume weight	1.39	1.26	1.28	1.28	1.20	1.05
Spread	.06	.25	.16	.14	.20	.28

SECTION C

Trials, number	10 (No. 1)	10 (No. 2)	10 (No. 2)	10 (No. 2)	10 (No. 2)	10 (No. 2)
Average volume weights	1.43±0.005	1.44±0.006	1.36±0.012	1.35±0.014	1.36±0.008	1.30±0.042
Standard deviation	.0235	.0265	.0574	.0648	.0361	.1900
Coefficient of variation, per cent	1.64	1.84	4.22	4.80	2.65	15.31
Highest volume weight	1.45	1.49	1.43	1.41	1.40	1.86
Lowest volume weight	1.39	1.36	1.23	1.17	1.29	1.05
Spread	.06	.13	.20	.24	.11	.81

[a] Formula used:

Standard deviation, $SD=\sqrt{\frac{\Sigma d^2}{n}}$

Coefficient of variation, $CV=\frac{SD\times 100}{m}$. d=deviation; n=number of items; m=mean.

Probable error of mean$=\frac{0.6745\times SD}{\sqrt{n}}$

A comparison of the three sections of this table indicates that there is little, if any, difference between the improved soil-tube method, the sand method, and the rubber-tube method so far as consistency of the data is concerned; and apparently a correction factor should be applied, to secure correct results, where any one of these methods is used. Since sections B and C show some differences in the results from duplicate sets of holes, it appears that at least 10 determinations should be made on soils of this type to obtain reliable results.

After the tests described above were finished and the calculations made, it was thought wise to make further experiments with the sand method. Apparently this method is as accurate and reliable as any except the cylinder method, and it can be used in places where the improved soil-tube method is not practical. Therefore, additional tests for 2, 3, and 4 feet were made in order to determine the correction factor for these depths.

A comparison of the results from all tests with the sand and cylinder methods for the same depths is presented in Table 3. The correction factors shown were computed in the same manner as the correction factor described above. The factors for depths of 3, 4, and 5 feet are about the same, but the one for the first 2 feet is a little large, for no apparent reason.

TABLE 3.—*Individual soil volume-weight determinations made by cylinder and sand methods at various depths*

[Second group of tests]

Group No.	Hole No.	Volume weights, for—							
		First 2 feet by—		First 3 feet by—		First 4 feet by—		First 5 feet by—[a]	
		Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method
1	1	1.46	1.41	1.48	1.48	1.45	1.39	1.45	1.48
1	2		1.45		1.39		1.38		1.48
2	1	1.43	1.55	1.44	1.48	1.44	1.43	1.45	1.26
2	2		1.50		1.29		1.44		1.36
3	1	1.44	1.49	1.44	1.54	1.41	1.49	1.42	1.51
3	2		1.49		1.41		1.47		1.49
4	1	1.45	1.37	1.46	1.49	1.45	1.48	1.45	1.40
4	2		1.34		1.48		1.51		1.45
5	1	1.43	1.40	1.43	1.52	1.43	1.33	1.45	1.46
5	2		1.48		1.44		1.44		1.47
6	1	1.42	1.47	1.43	1.50	1.43	1.46	1.44	1.45
6	2		1.37		1.42		1.39		1.40
7	1	1.40	1.26	1.40	1.46	1.40	1.32	1.42	1.45
7	2		1.31		1.45		1.50		1.48
8	1	1.37	1.42	1.38	1.29	1.39	1.37	1.41	1.39
8	2		1.45		1.34		1.36		1.44
9	1	1.36	1.44	1.37	1.29	1.37	1.34	1.39	1.42
9	2		1.28		1.31		1.37		1.43
10	1	1.30	1.35	1.33	1.38	1.36	1.37	1.39	1.38
10	2		1.40		1.44		1.36		1.44
Average		1.41 ±0.010	[b]1.41 ±0.011	1.42 ±0.009	[b]1.42 ±0.012	1.41 ±0.007	[b]1.41 ±0.009	1.43 ±0.005	[b]1.43 ±0.008

[a] The figures in these two columns were taken from Table 1.

[b] The correction factor for the sand method is 0.9195 for the first 2 feet, 0.8800 for the first 3 feet, 0.8744 for the first 4 feet, and 0.8763 for the first 5 feet.

Comparisons of these data on the basis of 10 tests each are shown in Table 4 and divided into three sections as in Table 2. Table 4 shows that the results obtained by the cylinder method are more consistent than those obtained by the sand method. However, the results with the sand method in this trial are consistent with those in the first. Apparently this method can be used with a fair degree of accuracy for at least 5 feet in depth.

TABLE 4.—*Comparisons between results obtained in soil volume-weight determinations for different depths, using the cylinder and sand methods*

SECTION A

	Values for the—							
	First 2 feet by—		First 3 feet by—		First 4 feet by—		First 5 feet by—	
	Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method	Cylinder method	Sand method
Trials, number	10	20	10	20	10	20	10	20
Average volume weight	1.41 ±0.010	1.41 ±0.011	1.42 ±0.009	1.42 ±0.012	1.41 ±0.007	1.41 ±0.009	1.43 ±0.005	1.43 ±0.006
Standard deviation	.0471	.0755	.0434	.0781	.0308	.0583	.0235	.0557
Coefficient of variation, per cent	3.34	5.35	3.06	5.50	2.18	4.13	1.64	3.90
Highest volume weight	1.46	1.55	1.48	1.54	1.45	1.51	1.45	1.51
Lowest volume weight	1.30	1.26	1.33	1.29	1.36	1.32	1.39	1.26
Spread	.16	.29	.15	.25	.09	.19	.06	.25

SECTION B

Trials, number	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10 (No.1)	10(No. 1)
Average volume weight	1.41 ±0.010	1.42 ±0.016	1.42 ±0.009	1.44 ±0.018	1.41 ±0.007	1.40 ±0.013	1.43 ±0.005	1.42 ±0.010
Standard deviation	.0471	.0762	.0434	.0866	.0308	.0600	.0235	.0469
Coefficient of variation, per cent	3.34	5.37	3.06	6.01	2.18	4.29	1.64	3.30
Highest volume weight	1.46	1.55	1.48	1.54	1.45	1.49	1.45	1.51
Lowest volume weight	1.30	1.26	1.33	1.29	1.36	1.32	1.39	1.26
Spread	.16	.29	.15	.25	.09	.17	.06	.25

SECTION C

Trials, number	10 (No.1)	10 (No.2)	10 (No.1)	10 (No.2)	10 (No.1)	10 (No.2)	10 (No.1)	10(No. 2)
Average volume weight	1.41 ±0.010	1.41 ±0.016	1.42 ±0.009	1.40 ±0.013	1.41 ±0.007	1.42 ±0.012	1.43 ±0.005	1.44 ±0.006
Standard deviation	.0471	.0748	.0434	.0600	.0308	.0548	.0235	.0265
Coefficient of variation, per cent	3.34	5.30	3.06	4.29	2.18	3.86	1.64	1.84
Highest volume weight	1.46	1.50	1.48	1.48	1.45	1.51	1.45	1.49
Lowest volume weight	1.30	1.28	1.33	1.29	1.36	1.36	1.39	1.36
Spread	.16	.22	.15	.19	.09	.15	.06	.13

SUMMARY

This paper reports a comparative study of five different methods of determining soil-volume weights. These tests were made on Gila clay adobe to a depth of 5 feet, and the conclusions are based on the study of only this particular soil.

Ten determinations were made with the cylinder method, 20 with the viscous-fluid method, 20 with the rubber-tube method, 40 with the improved soil-tube method, and 80 with the sand method. In all, 170 determinations were made in an area about 20 by 2½ feet.

The cylinder method gave reliable and satisfactory results.

The viscous fluid method gave such variable results that it can not be recommended for such conditions as prevailed in these experiments.

The sand, rubber-tube, and improved soil-tube methods rank about the same so far as the variability of the results is concerned. To secure accurate results with these methods, a correction factor should be used.

PERCENTAGE DRY MATTER AND FIELD WEIGHT OF EAR CORN FROM UNLIMED AND LIMED PLOTS[1]

By A. W. Blair

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In connection with the field studies on the availability of nitrogen that have been carried on at this station for the past 22 years, corn has been one of the crops in a 5-year rotation. The experiment provides for twenty 1/20-acre plots with different nitrogen treatments, without lime, and twenty corresponding plots with lime. The lime material has been either ground oyster shells or ground limestone, and has been applied at the rate of 2 tons per acre once in five years. This has been sufficient to maintain the hydrogen-ion concentration of the soil at close to pH 6.8 to 7.0. The majority of the unlimed plots maintain a fairly constant pH value of about 5.2 to 5.3. A few, however, have a pH of 5.8 and one has a pH of 6.0.

The soil is a sassafras loam of fair quality. What treatment it received before the plots were laid out in 1908 is not known, but it is very probable that no lime had been used for many years.

Corn was grown in 1908, 1913, 1918, 1923, and 1928. The other crops in the rotation were oats, wheat, and for two years, timothy.

In preparing the samples for analysis, the percentage of dry matter in the ear corn was determined. The figures for 1918, 1923, and 1928, together with field weights of ear corn, were available and are shown in Table 1. In 1918 the corn from 18 of the 20 plots treated with lime showed a higher percentage of dry matter than the corn from plots without lime; in 1923 the corn from 16 of the 20 limed plots showed a higher percentage of dry matter than the corn from the corresponding unlimed plots, while in 1928 the corn from 17 of the 20 limed plots showed a higher percentage of dry matter than the corn from the corresponding unlimed plots. These differences are clearly brought out in the averages, the average difference being a little more than 2 per cent in 1918 and about 4 per cent in 1923 and 1928. This difference in content of dry matter in favor of the corn from the limed plots would seem to indicate that corn grown on acid soil does not mature and dry out quite so early as corn grown on the same soil with a fair supply of lime. Other experiments have shown that corn on very acid soil is inclined to remain green longer than that grown on well-limed soils. In the work reported here, however, it was only the corn on a few of the most acid plots that exhibited a difference that could be detected with the eye, but apparently the well-limed soil slightly speeded up maturity. Corn on these plots is usually harvested during the last half of September, and that date is not too early for maturity, if growing conditions have been favorable.

The field weight of ear corn is shown along with the percentage of dry matter. The general averages show some difference in favor of the limed plots. If, however, the weights for certain unlimed plots

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which have received extreme treatment should be omitted from the average, the difference between the yields from the limed and unlimed plots would be slight. It would appear, therefore, that in this case the effect of the lime has been in the direction of uniform ripening and drying of the corn rather than in increased weight. The unfavorable conditions which frequently exist in acid soils might account for the retarded maturity.

TABLE 1.—*Field weight of ear corn (acre basis) and percentage dry matter in ear corn from unlimed and limed plots*

Plot No.	Fertilizer treatment (per acre)	Field weight and percentage dry matter in ear corn raised in—											
		1918				1923				1928			
		Without lime		With lime		Without lime		With lime		Without lime		With lime	
		Pounds	Per cent	Pounds	Per cent	Pounds	Per cent	Pounds	Per cent	Pounds	Per cent	Pounds	Per cent
1	Nothing	4,380	63.29	3,850	65.38	3,100	67.18	3,475	71.29	1,900	64.21	1,575	71.43
2	160 pounds muriate of potash	3,720	66.99	4,050	66.70	2,500	60.20	4,300	73.95	1,525	65.57	2,275	69.78
3	320 pounds superphosphate	4,140	67.54	3,790	69.90	3,650	64.32	3,250	72.15	1,750	68.29	1,275	70.59
4	Minerals only [a]	3,360	67.02	3,720	70.54	3,225	65.66	4,000	72.25	1,550	66.13	1,775	73.24
5	Minerals and 16 tons cow manure	4,420	65.88	3,990	70.74	4,550	70.49	3,700	75.07	4,400	72.16	2,750	71.36
6	Minerals only	4,760	68.91	4,350	71.14	5,200	68.08	5,075	73.35	4,150	71.39	2,375	74.74
7	Nothing	2,340	68.38	3,910	69.84	1,350	63.70	3,750	71.07	82	69.51	875	70.00
8	Minerals and 160 pounds $NaNO_3$	3,400	68.71	4,010	70.06	3,000	63.50	4,525	71.22	1,650	69.70	2,400	73.44
9	Minerals and 320 pounds $NaNO_3$	4,120	69.81	3,970	68.35	3,625	69.86	3,800	69.67	2,600	70.67	2,150	73.84
10	Minerals and $Ca(NO_3)_2$ equivalent to 320 pounds $NaNO_3$	4,020	66.27	4,700	70.04	3,800	62.70	5,000	72.25	2,350	69.36	3,050	73.77
11	Minerals and $(NH_4)_2SO_4$ equivalent to 320 pounds $NaNO_3$	1,340	67.46	4,560	70.00	1,450	62.93	4,550	71.65	76	71.05	2,150	73.84
12	Minerals and $CaCN_2$ equivalent to 320 pounds $NaNO_3$	4,320	68.61	3,880	70.62	4,425	68.87	5,000	69.55	2,625	76.67	3,900	75.64
13	Minerals only	4,480	66.61	3,820	69.95	4,650	66.45	4,900	70.31	900	69.44	2,875	80.87
14	Minerals and $NaNO_3$ $(NH_4)_2SO_4$ (N, half and half)	4,500	68.98	3,920	70.41	4,875	71.74	4,600	74.24	2,900	66.81	3,500	73.93
15	Minerals and tankage equivalent to 320 pounds $NaNO_3$	3,900	67.90	4,080	73.04	3,825	74.58	4,425	73.33	3,350	68.06	2,850	69.74
16	Minerals only	4,820	70.04	4,140	70.24	3,325	74.62	4,525	75.86	2,275	68.13	2,350	76.06
17	do	3,300	68.36	5,000	72.56	2,125	71.76	4,325	72.25	1,125	72.22	3,350	74.63
18	Minerals, 16 tons manure, and 320 pounds $NaNO_3$	4,720	70.25	5,120	70.94	5,150	72.14	5,550	71.76	5,175	74.88	4,825	76.17
19	Minerals only	3,040	69.34	4,160	73.17	2,675	72.15	3,825	73.79	1,325	67.92	2,375	75.79
20	Minerals, 2 tons rye straw, and 320 pounds $NaNO_3$	4,610	68.23	4,870	70.03	4,525	70.99	3,825	65.03	2,008	74.71	2,750	74.55
	Average	3,885	67.93	4,195	70.18	3,601	68.10	4,320	72.00	2,186	69.84	2,571	73.67

[a] Minerals=160 pounds muriate of potash and 320 pounds superphosphate.

In the eastern part of the United States there are millions of acres of land that have not been limed in many years, and it seems entirely possible that in the case of much of this land, acidity may have become one of the limiting factors in the successful growing of corn as well as of many other crops.

SUMMARY

Analyses of ear corn grown on sassafras loam of fair quality, fertilized in different ways, indicate that corn grown on acid soil does not mature and dry out quite so early as corn grown on the same soil with a fair supply of lime. General averages also indicate some difference in favor of the limed plots when the field weight of ear corn is considered, but if the weights for certain unlimed plots that received extreme treatment are omitted from the averages, the differences in yields between the limed and the unlimed plots are slight. Figures for corn grown in 1918, 1923, and 1928 are summarized.

CORRELATED INHERITANCE IN A CROSS (SEVIER × DICKLOW) × DICKLOW WHEATS[1]

By GEORGE STEWART, *Agronomist*, and R. K. BISCHOFF, *Graduate Student*, *Department of Agronomy, Utah Agricultural Experiment Station*

INTRODUCTION

This paper reports a study of the inheritance, particularly the correlated inheritance, of certain observed and measured plant characters in a wheat cross between a segregate from Dicklow × Sevier (F22), and a pure line from Dicklow (D3), one of the original parents of F22.

Since Hayes and Garber (*4*),[2] Clark (*1*), and Stewart (*6*) have recently compiled bibliographies of literature relating to the inheritance of the characters in wheat herein reported, literature citations are limited to those that refer directly to the material studied.

DESCRIPTION OF PARENTS

F22, true breeding for the characters studied, is a segregate from the cross Sevier × Dicklow, developed at the Utah station. The straw is much stronger than that of the Sevier parent, but not so strong as that of the Dicklow parent. The glumes are light bronze in color. It is a fully awned wheat, with awns 60 to 70 mm. long.

Measurements of the parent rows of F22 are summarized as follows:

Length of longest culm to base of spike	108. 43 ± 2. 06 cm.
Spike density—length of 10 rachis internodes	25. 61 ± . 86
Number of culms per plant	11. 61 ± . 84

Dicklow is a favorite spring wheat on the irrigated farms of Utah. Its popularity is due to its ability to resist lodging under irrigation and to produce a high yield. It has a medium tall, stiff straw. The lemmas near the apex of the spike bear short awns from 3 or 4 to 10 mm. in length. The glumes are white.

Measurements of the Dicklow parent rows follow:

Length of longest culm to base of spike	103. 84 ± 1. 80 cm.
Spike density—length of 10 rachis internodes	50. 3 ± . 577
Number of culms per plant	9. 43 ± 1. 46

These measurements indicate that F22 has a culm length averaging 4.59 ± 2.74 cm. longer than Dicklow. The difference is less than twice the probable error. The range for the longest culm is 92.5 to 123 cm. for F22, and from 93.5 to 117.4 for Dicklow. In spike density Dicklow, ranging from 43.47 to 64.75 cm. for 10 internodes, is nearly twice as lax as the F22 parent whose range is from 23.83 to 28.05 cm. The two parents differ but slightly in number of culms produced. F22 produces a mean of 11.61 ± 0.84 culms, with a range from 9.3 to 15.7 per plant. Since the Dicklow parent has a mean of 9.43 ± 1.46 ranging from 7.7 to 11.4 per plant, there is no real difference between the parents in this respect.

[1] Received for publication Nov. 7, 1930; issued June, 1931. Contribution from the Department of Agronomy, Utah Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 790.

EXPERIMENTAL PROCEDURE

The cross between a pure strain of F22 and one of Dicklow (D3) was made in 1925 at Logan, Utah. The F_1 plants were grown in 1926 and the F_2 families in 1927. One of the most vigorous of the F_2 families was chosen to continue the study. This family consisted of 257 plants, of which 51 were dwarfs. Of the 51 dwarfs 11 produced either no seed or not enough with which to propagate F_3 progenies. Each plant, whose development permitted it, was classified according to awn class, glume color, and spike density. The spike-density figure was obtained by measuring the length of 10 rachis internodes. Grain from each F_2 plant seeded an F_3 progeny row in 1928. The kernels were spaced about 3 inches apart, with 40 to 60 seeded in a row except where the F_2 plant furnished fewer kernels. The rows were 1 foot apart.

After each tenth progeny row the parental varieties, F22 and Dicklow, were sown side by side in the same manner and at the same time as the progeny rows. In all, there were 25 parent rows. Two of the Dicklow parent rows were destroyed by birds and are not included in the calculations. This planting arrangement made it possible to study the progeny characters in relation to the parental characters. One progeny row was also destroyed by birds.

When the grain was mature each F_3 progeny and each parent row was carefully harvested by pulling the plants individually. The plants of each row were bundled together, tied, and labeled. The material was worked in the laboratory during the winter months, except that awn data were taken in the field for the progenies with true-breeding awns. Culm length for the 237 rows in which there were 10 or more normal plants was measured by placing the root end of the individual plant against a footboard and extending the culms along a table board marked in centimeters. The longest culm was measured to the base of the spike and recorded. The number of dwarfs in all of the 245 progenies was determined by counting, which, with 11 F_2 dwarfs without progenies, makes 256 for F_2 plants classified for this character. In all other cases the 237 progeny rows with 10 or more normal plants were used except for awns, the data for which were secured on one additional progeny later destroyed by birds. The culms were counted with the precaution of avoiding second-growth culms; these frequently appeared at the base of the plant. Each plant was examined for its awn characteristics. Those that approached the F22 parent as determined by visual comparison with parent plants were classified as belonging to awn class 4, or simply to "awns 4." Those that approached the Dicklow parent were called "awns 2." Progenies that produced both awns–2 plants and awns–4 plants were said to be segregating. Spike density was determined by measuring 10 rachis internodes in the middle of a typical spike on each plant. This avoided the extremes of laxity and density found on opposite ends of the spike. Each plant was classified for glume color as white or bronze, determined by inspection.

The data were so taken and recorded that all data from each plant could be traced readily to that plant and to the row from which it came. This permitted the study of correlations.

The usual number of plants in each progeny was 40 to 45. In some progenies there were fewer than this, and in others there were more.

The parental rows consisted of approximately the same number of plants as did the progeny rows. No theory of inheritance was considered until all data were taken, recorded, and studied.

After the data were assembled and recorded, classifications and calculations were made. The mean length of the longest culm of all plants not classified as dwarfs was calculated for each progeny whether true breeding or not. The same calculation was made for the number of culms. The progeny rows were classified by inspection into segregating and true-breeding groups for stature, for awns, and for glume color. The mean spike density of each progeny row and of each parental row was obtained as well as the coefficient of variability (C. V.) for each row. When correlation studies were made the mean values for the F_3 progenies were the figures used. In other words, the F_2 plants were classified by the breeding behavior of their respective F_3 progenies. This was regarded as a more accurate indication of the genetic composition of the F_2 plants than was the individual character of the F_2 plants themselves.

EXPERIMENTAL RESULTS AND THEIR INTERPRETATION

Inheritance studies were made of individual plant characters, and correlation studies were made between various pairs of characters.

INHERITANCE OF INDIVIDUAL CHARACTERS

Inheritance studies were made for the following individual plant characters: Culm length, stature, number of culms, awn classes, spike density, and color of glume.

TABLE 1.—*Parental rows and F_3 progenies arranged according to means of culm length and according to the coefficients-of-variability (C. V.) classes*

[Cross 35c; grown in 1928 at Logan, Utah. Length of longest culm in centimeters, normal plants]

Parent or progeny	Number of progenies of culm length indicated										Total	C. V. class
	85	90	95	100	105	110	115	120	125	130		
											Number	*Per cent*
Dicklow				1	2	4	2				9	4
			4	4	3	1					12	8
			1								1	12
			1								1	16
Total or mean											23	6.94
F22						1	1				2	4
			1	1	8	1	4	2	1		18	8
			2	1							3	12
			1				1				2	16
Total or mean											25	8.46
F_3 progenies			3	6	8	7	12	7	2		45	4
	1	2	9	39	29	30	20	12	2	2	146	8
	1	4	8	11	4	6	2	4			40	12
			1	1	1						3	16
				2	1						3	18
Total or mean											237	7.95

Culm Length

In Table 1 are compared the distribution of normal plants in the F_3 progenies having 10 or more normal plants for culm length and its respective coefficient of variability, and the same data for the parental rows. The range for F22 parents is from 92.5 to 123 ± 6.06 cm.; that of the Dicklow parent from 93.5 to 117.4 ± 4.80 cm. The ranges and the mean heights of the two parents are similar. The range in culm length from the F_3 progenies (fig. 1) is from 88.0 to 128.0 ± 5.77 cm. There were about 10 times as many individuals considered in the progenies as there were in the parental rows of each parent. The distribution shown fails to indicate segregating groups.

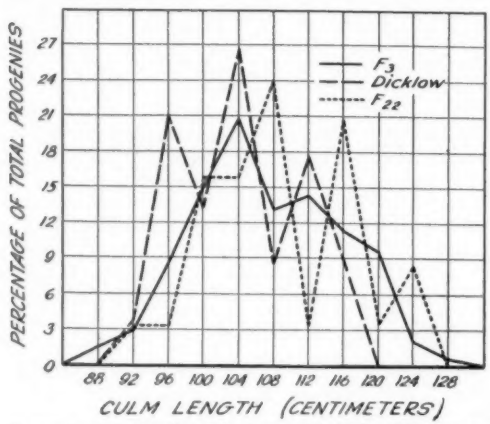

FIGURE 1.—Culm lengths of the two parents, F22 and Dicklow, and of the normal plants in all the F_3 progenies. This character was widely variable, due to soil heterogeneity. After allowance is made for this variability there is no measurable difference between the two parents or between the parents and the F_3 progenies.

Stature

The F_3 progenies segregated for normal and dwarf stature as follows: 98 were segregating for either 3 normal to 1 dwarf or 13 normal to 3 dwarf plants; 29 progenies were segregating 1 normal to 3 dwarf plants; 11 progenies were homozygous for the dwarf character, and 107 were homozygous for normal stature. Both the number of F_2 plants and the breeding behavior of the F_3 progenies suggest a 13:3 ratio for normal and dwarf segregation in F_2.

Two factors are involved in this explanation of dwarfness: (1) *Ii*, an inhibitor factor which prevents the expression of dwarfness when the dominant *I* is present either in the heterozygous or in the homozygous condition; and (2) *Dd*, a dwarf factor assumed to be a dominant, which produces dwarfs when in either the homozygous or the heterozygous condition unless the inhibiting factor is present as *II* or as *Ii*.

The recombination of these factors would produce all normal plants in F_1 but would call for two phenotypes in F_2, normal and dwarf, whose F_2 characters and whose F_3 breeding behavior might be summarized as follows:

P_o F22 *IIDD* × Dicklow *iidd*.
F_1 *IiDd*.
F_2 13 normal: 3 dwarf.

The 13 normal F_2 plants would have the following genotypic composition and F_3 breeding behavior:

1 *IIDD* (normal in F_2, normal in F_3).
2 *IIDd* (normal in F_2, normal in F_3).
2 *IiDD* (normal in F_2, segregate 3 normal: 1 dwarf in F_3).
4 *IiDd* (normal in F_2, segregate 13 normal: 3 dwarf in F_3).
1 *IIdd* (normal in F_2, normal in F_3).
2 *Iidd* (normal in F_2, normal in F_3).
1 *iidd* (normal in F_2, normal in F_3).

The three dwarf F_2 plants would have the following genotypic composition and F_3 breeding behavior:

1 *iiDD* (dwarf in F_2, dwarf in F_3).
2 *iiDd* (dwarf in F_2, segregate 3 dwarf: 1 normal in F_3).

TABLE 2.—*Segregation for normal and dwarf plants in F_3 progenies from normal F_2 plants, grouped into three classes determined by the nature of the segregation of normal to dwarf plants: 13 : 3, 3 : 1, and 1 : 3*

[Family 35c; grown in 1928 at Logan, Utah]

CALCULATED SEGREGATION BASED ON 13 : 3

Normal plants	Dwarfs		Deviation calculated	P. E.	Dev./P. E.
	Found	Calculated			
Number	*Number*	*Number*			
27	5	6	1	1.49	0.67
38	4	7.9	3.9	1.71	2.27
31	4	6.6	2.6	1.56	1.66
31	5	6.8	1.8	1.58	1.13
40	1	7.7	6.7	1.69	3.96
25	1	4.9	3.9	1.34	2.91
32	3	6.7	3.7	1.56	2.36
34	7	7.7	.7	1.69	.41
23	3	4.9	1.9	1.34	1.41
44	6	9.4	3.4	1.86	1.83
34	5	7.3	2.3	1.64	1.40
37	6	8.06	2.06	1.73	1.19
27	1	5.3	4.3	1.39	3.09
36	6	7.9	1.9	1.71	1.11
31	5	6.8	1.8	1.58	1.13
32	2	6.4	4.4	1.54	2.85
24	3	5.01	2.01	1.37	1.46
40	6	8.6	2.6	1.79	1.45
40	3	8.06	5.06	1.73	2.92
36	5	7.7	2.7	1.69	1.59
25	3	5.3	2.3	1.39	1.65
29	2	5.8	3.8	1.47	2.58
37	7	8.3	1.3	1.75	.74
27	6	6.2	.2	1.51	.13
32	5	6.9	1.9	1.60	1.18
38	5	8.1	3.1	1.73	1.79
33	8	7.7	.3	1.69	.17
40	3	8.1	5.1	1.73	2.94
33	4	6.9	2.9	1.60	1.81
34	7	7.7	.7	1.69	.41
40	3	8.1	5.1	1.73	2.94
27	3	5.6	2.6	1.44	1.80
35	5	7.5	2.5	1.66	1.50
25	5	5.6	.6	1.44	.41
37	9	8.6	.4	1.79	.22
37	9	8.6	.4	1.79	.22
40	14	10.1	3.9	1.93	2.02
28	6	6.4	.4	1.54	.25
40	10	9.4	.6	1.86	.32
27	7	6.4	.6	1.54	.38
22	6	5.3	.7	1.55	.45
35	8	8.1	.1	1.73	.06
40	10	9.4	.6	1.86	.32
29	9	7.1	1.9	1.62	1.17
34	8	7.9	.1	1.71	.06
40	11	9.6	1.4	1.88	.74
40	8	9.0	1.0	1.82	.54
36	8	8.3	.3	1.75	.17
25	6	5.8	.2	1.47	.14
40	10	9.4	.6	1.86	.32
32	8	7.5	.5	1.66	.30
40	9	9.2	.2	1.64	.12
44	14	10.9	.5	2.15	.23

TABLE 2.—*Segregation for normal and dwarf plants in F_3 progenies from normal F_2 plants, grouped into three classes determined by the nature of the segregation of normal to dwarf plants: 13 : 3, 3 : 1, and 1 : 3*—Continued

CALCULATED SEGREGATION BASED ON 3:1

Normal plants	Dwarfs		Deviation calculated	P. E.	Dev./P. E.
	Found	Calculated			
Number	*Number*	*Number*			
33	10	10.8	0.8	1.92	0.41
34	13	11.8	1.2	2.00	.60
34	14	12.0	2.0	2.02	.99
33	15	12.0	3.0	2.02	1.48
27	16	10.8	5.2	1.92	2.70
24	10	8.5	1.5	1.70	.88
32	10	10.5	.5	1.89	.26
38	11	12.3	1.3	2.04	.63
28	17	11.3	5.7	1.96	2.90
38	13	12.8	.2	2.09	.09
28	16	11.0	5.0	1.94	2.57
34	14	12.0	2.0	2.02	.99
37	11	12.0	1.0	2.02	.49
29	11	10.0	1.0	1.85	.54
40	15	13.8	1.2	2.17	.55
31	13	11.0	2.0	1.94	1.03
28	13	10.3	2.7	1.87	1.44
31	9	10.0	1.0	1.85	.54
27	12	9.8	2.2	1.82	1.20
27	10	9.3	.7	1.78	.39
20	8	7.0	1.0	1.55	.64
31	16	11.8	4.2	2.00	2.10
37	11	12.0	1.0	2.02	.49
27	14	10.3	3.7	1.87	1.97
19	9	7.0	2.0	1.55	1.29
29	10	9.8	.2	1.82	.10
24	13	9.3	3.7	1.78	2.07
35	10	11.3	1.3	1.96	.66
29	13	10.5	2.5	1.89	1.32
24	7	7.8	.8	1.63	.49
23	7	7.5	.5	1.60	.31
36	12	12.0	.0	2.02	.00
26	10	9.0	1.0	1.75	.57
38	14	13.0	1.0	2.11	.47
40	14	13.5	.5	2.15	.23
22	8	7.5	.5	1.60	.31
29	12	10.3	1.7	1.87	.90
25	11	9.0	2.0	1.75	1.15
24	10	8.5	1.5	1.70	.88
37	12	12.3	.3	2.04	.15
39	14	13.25	.8	2.13	.37
27	13	10.0	3.0	1.85	1.62
19	7	6.5	.5	1.49	.33
29	11	10.0	1.0	1.85	.54
27	15	10.5	4.5	1.89	2.38

CALCULATED SEGREGATION BASED ON 1:3

15	25	30.0	5.0	1.85	2.70
15	16	23.2	7.2	1.63	4.42
19	25	33.0	8.0	1.94	4.12
12	31	32.2	1.2	1.92	.62
15	36	38.2	2.2	2.09	1.05
7	23	22.5	.5	1.60	.31
22	30	39.0	9.0	2.11	4.27
11	38	36.7	1.3	2.04	.64
18	33	38.2	5.2	2.09	2.49
14	24	28.5	4.5	1.80	2.50
10	26	27.0	1.0	1.75	.57
10	25	26.2	1.2	1.73	.69
9	30	29.2	.8	1.82	.44
12	42	40.5	1.5	2.15	.70
15	26	30.7	4.7	1.87	2.51
12	34	34.5	.5	1.98	.25
14	36	37.5	1.5	2.07	.72
14	37	38.2	1.2	2.09	.57
10	34	33.0	1.0	1.94	.52
9	24	24.7	.7	1.68	.42
10	37	35.2	1.8	2.00	.90
7	40	35.2	4.8	2.00	2.40
25	35	45.0	10.0	2.26	4.42
16	38	40.5	2.5	2.15	1.16
13	32	33.7	1.7	1.96	.87
13	25	28.5	3.5	1.80	1.94
5	31	27.0	4.0	1.75	2.29
28	42	52.5	10.5	2.44	4.30
7	33	30.0	3.0	1.85	1.62

It was impossible by inspection to separate the 13:3 and the 3:1 segregating groups; therefore, the separation was attempted by the Dev./P. E. method and is summarized in Table 2. The Dev./P. E. calculation was made for each progeny considered on the basis of a 13:3 segregation and also on the basis of a 3:1 segregation. The group segregating 1 normal:3 dwarf was also included in the calculation. The final separation was made on the basis of the smallest Dev./P. E. calculation. By this calculation 45 progenies were found to be segregating for the 3:1 ratio; 53 for the 13:3 ratio; and 29 for the 1:3 ratio. There were 11 progenies homozygous for dwarf stature and 107 homozygous for normal stature. In addition, there were 11 F_2 dwarfs which produced no F_3 progenies. Forty other phenotypically similar F_2 plants of dwarf stature produced 29 progenies segregating 1 normal:3 dwarf, and 11 progenies breeding true for dwarf stature. If the 11 F_2 dwarfs without progeny be assumed to segregate in the same proportions, this would mean that 3 of the 11 would have been true-breeding for dwarf stature and that 8 would have segregated 1 normal:3 dwarf.

The Dev./P. E. method of separating progenies was effective in separating the 1:3 group from the other two. The 13:3 and the 3:1 groups were not satisfactorily separated, perhaps on account of the unequal number (twice as many in the 13:3 group) of overlapping variants from two groups so closely similar in the nature of their segregation. The closeness-of-fit study would have been more satisfactory had it been possible to make a complete separation between the 13:3 and the 3:1 segregating groups. Since, however, this could not be done with the data available, the two groups were left together. By this grouping there were 98 progenies segregating 13:3 or 3:1, and 29 progenies segregating 1:3 to which must be added 8, the proportionate share of the 11 F_2 dwarf plants which had no progeny, leaving 3 to be classified as true dwarf. This makes 37 progenies in the 1:3 group, and 14 in the true-breeding dwarf group (11 with true-breeding dwarf progenies plus 3 from the 11 with no progenies). In order to see how well the theory fits the facts, X^2 and P were calculated in Tables 3 and 4. Table 3, with the 13:3 and the 3:1 groups split, shows a poor fit, $P=0.08$, but since all the calculated groups are close to the observed, save the 13:3 and the 3:1, Table 4 gives the closeness of fit with these two expected groups regarded as one, which in this case more nearly approximates the actual data.

TABLE 3.—*Closeness of fit of five groups of progenies on a basis of 13 : 3 F_2 segregation*

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
Homozygous normal	107	112	−5	25	0.2232
Segregating 13:3	53	64	−11	121	1.8906
Segregating 3:1	45	32	13	169	5.2813
Segregating 1:3	37	32	5	25	.7813
Homozygous dwarf	14	16	−2	4	.2500

$X^2=8.4264$. $P=0.0786$.

TABLE 4.—*Closeness of fit of four groups of progenies on a basis of 13 : 3 F_2 segregation*

[Groups 13:3 and 3:1 not separated; family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
Homozygous normal	107	112	−5	25	0.2232
Segregating 13:3 and 3:1	98	96	2	4	.0416
Segregating 1:3	37	32	5	25	.7813
Homozygous dwarf	14	16	−2	4	.2500

$X^2=1.2961$. $P=0.7335$.

As applied in Table 4, the theory fits the observed data so well that in 73 cases out of 100 a wider deviation might be expected, due to chance alone. The agreement of the theory with the data from the F_2 plants (expected dwarfs, 48.2; obtained, 51.0; deviation, 2.8; probable error, 4.22) and with the data from the F_3 progenies indicates that the theory of one dominant dwarfing factor *Dd* and an inhibitor *Ii* probably correctly explains the results obtained.

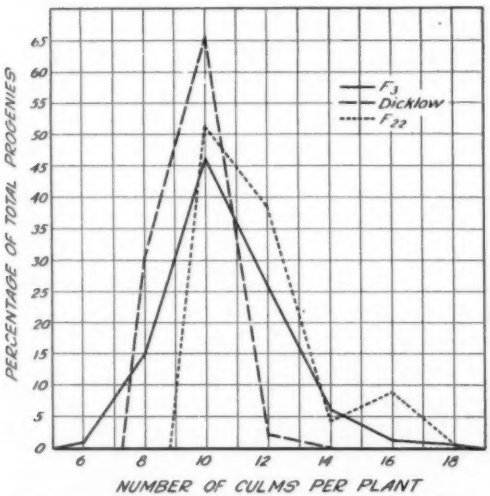

FIGURE 2.—Number of culms for the two parents, F22 and Dicklow, and for the F_3 progenies. This character varied widely, due to soil heterogeneity. No measurable differences were found between the parents or between the parents and the F_3 progenies

NUMBER OF CULMS

As judged by their probable errors, the F22 and Dicklow parents are essentially alike in the number of culms produced, being 11.61 ± 0.84 and 9.43 ± 1.46, respectively. There seems to be some segregation, but the environmental variability as shown in Table 5 is so high as to hide its nature. The mean coefficients of variability are almost identical for both parents and for the F_3 progenies. Both parental types were recovered, as the curves in Figure 2 show.

AWN CLASSES

In summarizing various inheritance studies, Hayes and Garber (*4*) found awn inheritance to be simple in crosses between some varieties and in others more complex. They concluded that there may be one or two or more genetic factors involved for awns. Howard and Howard (*5*) obtained single-factor results in some crosses, but in others between fully awned and awnless parents they were able to separate the F_2 progenies into five or six classes, which results required two factors to explain.

TABLE 5.—*Parental rows and F_3 progenies arranged according to number of culm classes and according to coefficient-of-variability (C. V.) classes*

[Cross 35c; grown in 1928 at Logan, Utah]

Parent or progeny	Number of progenies in culm class indicated												Total	C. V. classes
	6	7	8	9	10	11	12	13	14	15	16	17		
													Number	*Per cent*
Dicklow		1	1	2	1								5	30
			2	3	3	1							9	40
		1	2	2	2								7	50
			1		1								2	60
Total or mean													23	42.48
F22					2					1			3	30
				2	5	3	3		1				14	40
				1	2	1				1			5	50
					1		2						3	60
Total or mean													25	44.45
F_1				1			1						2	20
			3	11	4	6	4	1	3	2		1	35	30
	2	3	15	25	24	20	8	5	2		1	1	106	40
		3	4	15	21	12	5	5	2	1			68	50
			1	5	3	7	2	1					19	60
			3	1	1								5	70
			1	1									2	80
Total or mean													237	43.96

In this cross, the F22 parent is homozygous for fully developed awns (awns 4), and the Dicklow parent for tip awns (awns 2). There were 56 progenies homozygous for awns 2, 131 were heterozygous for awn inheritance, and 51 were breeding true for awns 4. When compared for closeness of fit on the basis of a 1:2:1 segregation, $\chi^2=2.6303$ and $P=0.2766$. (Table 6.) This fit indicates that the segregation considered is probably correct since a worse fit would be expected in 28 cases out of every 100 due to chance alone. No indication was found as to the dominance or recessiveness of awns.

TABLE 6.—*Closeness of fit of three groups of F_3 progenies on a basis of 1:2:1 segregation for awns*

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value(O)	Calculated value(C)	O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
Homozygous awns 2	56	59.5	−3.5	12.25	0.2059
Heterozygous	131	119	12.0	144.00	1.2101
Homozygous awns 4	51	59.5	−8.5	72.25	1.2143

$\chi^2=2.6303$. $P=0.2766$.

SPIKE DENSITY

The mean spike densities and the coefficients of variability were calculated for each progeny. The F_3 progenies were classified into three groups: (1) Those homozygous for dense spikes; (2) those heterozygous for spike density, and (3) those homozygous for lax spikes.

Table 7 compares the spike-density classes in their coefficient-of-variability (C. V.) classes of both parents and F_3 progenies.

TABLE 7.—*Frequency distribution of the rows of the Dicklow and F22 parents and of F_3 progenies, arranged into classes according to the mean spike density and according to the coefficients-of-variability (C. V.) classes of the individual rows of parents and of the F_3 hybrid progenies*

[Family 35c; grown in 1928 at Logan, Utah; millimeters for 10 rachis internodes]

Strain	Number of progenies of mean spike density indicated												Total	C. V. classes
	20	24	28	32	36	40	44	48	52	56	60	64		
													Number	*Per cent*
F22		3											3	10
		15	6										21	14
		1											1	18
Total or mean													25	13.68
Dicklow								4	8				12	8
								5	2				7	12
							1	1	1				3	16
												1	1	20
Total or mean													23	10.54
Homozygous dense	1	1											2	6
	12	17											29	10
	6	16	3	2									27	14
			1										1	18
	1												1	22
Total or mean													60	12.00
Heterozygous							1						1	24
			1	2	4	1		1					9	28
		2	1	4	6	3	1						17	32
		1	3	15	12	6	2						39	36
		1	3	14	13	6							37	40
			1	8	7	2		1					19	44
			2	2	1	1							6	48
Total or mean													128	38.15
Homozygous lax											2		2	4
								1	3	15	7	1	27	8
									4	8	5	2	19	12
											1		1	16
Total or mean													49	9.04

TABLE 8.—*Frequency distribution of the F_3 progenies from a cross of Dicklow × F22, arranged into classes according to the mean spike density and according to coefficient-of-variability (C. V.) classes*

[Three classes: (1) Those homozygous for dense spikes; (2) those heterozygous; and (3) those homozygous for lax spikes are plotted in one table to show the well-defined grouping of the three classes. Family 35c; grown in 1928 at Logan, Utah; spike density for 10 internodes in millimeters]

Number of F_3 progenies in mean spike denisty class indicated												Total	C. V. classes
20	24	28	32	36	40	44	48	52	56	60	64		
												Number	*Per cent*
1	1									2		4	6
12	17		1				1	3	15	7	1	57	10
6	16	3	1				4	8	5	2		45	14
		1							1			2	18
1												1	22
						1						1	26
		1	2	4	1		1					9	30
	2	1	4	6	3	1						17	34
	1	3	15	12	6	2						39	38
	1	3	14	14	6							38	42
			8	7	2		1					18	46
		2	2	1	1							6	50
												237	

Heterozygosity or homozygosity was indicated by the size of the coefficient of variability. The mean coefficient of variability for the homozygous F22 parent was 13.68 ± 1.428, with a range from 10.24 to 16.25 per cent. The same calculation for Dicklow was 10.54 ± 1.018, with a range from 7.7 to 16.19 per cent. The mean coefficient of variability for the homozygous-dense group was 12.00 ± 0.739 per cent and ranged from 6.45 to 22.30; the lax group exhibited a mean of 9.04 ± 0.636 per cent and a range from 4.1 to 16.22; the heterozygous group had a mean of 38.15 ± 1.818 per cent with a range from 23.83 to 47.42 per cent for the same character. These figures indicate that the true-breeding F_3 rows are no more variable than the parental rows, but that the heterozygous are about three to four times as variable.

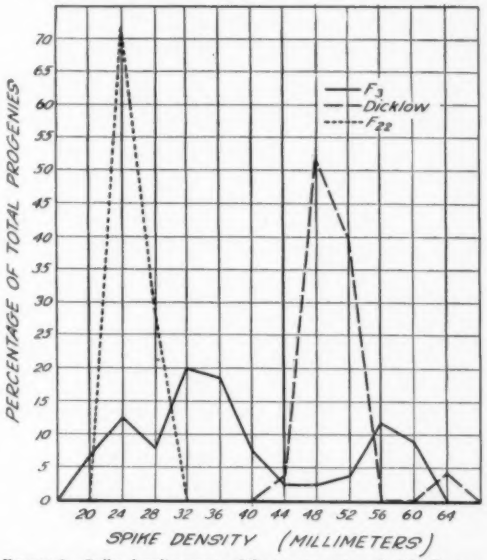

FIGURE 3.—Spike-density curves of the two parents and of the F_3 progenies. One parent, F22, is distinctly more dense than the other parent, Dicklow. In the F_3 progenies there were three definite classes: (1) Homozygous dense with a mean almost identical with F22; (2) homozygous lax which is statistically somewhat more lax than Dicklow; and (3) an intermediate heterozygous group. The three groups overlap somewhat and make a continuous curve but show three peaks, one for each major group. The plants in the homozygous groups were no more variable than the parents

Table 8 proves rather definitely that there were progenies which were homozygous for dense spikes, others which were homozygous for lax spikes, and that the spikes of intermediate length were heterozygous. The rows of homozygous-dense progenies tended to be similar in appearance and density to the F22 parent. The heterozygous group was intermediate between the parents and contained individuals representative of both parental groups, indicating segregation. The homozygous-lax progenies were similar to the Dicklow parent. In only three progenies was the mean density of the Dicklow spike recovered. The mean length of each rachis internode in the F_3 progenies was 56.5 ± 1.74 mm. as compared with 50.3 ± 0.577 mm. for Dicklow. The lax group have a greater length of 6.2 ± 1.83, which is three and four-tenths times its probable error. The figures indicate a tendency toward transgressive segregation in the direction of a more lax spike than that which characterizes the Dicklow parent. Figure 3 shows the spike density curves.

Table 9 indicates that the range of spike density and the range of coefficients of variability (C. V.) are not far different in homozygous groups of progenies than in either parent. It also proves that the density of the F22 parent was recovered almost identically. The

mean spike density of the F22 parent was 25.6 ± 0.861 as compared with a mean of 25.2 ± 0.707 for the homozygous-dense progenies. The heterozygous group could be identified at a glance by the high coefficients of variability. This definitely high coefficient of variability (38.15 ± 1.818) clearly designates the segregating progenies and lends additional evidence to the observation previously made that transgressive segregation probably occurred toward a more lax spike than that of the lax parent, which indicates some sort of additional modifying factor, not clearly isolated by their data. The homozygous-lax progenies, having a mean coefficient of variability of 10.54 ± 1.018 per cent, were easily distinguished from the segregating progenies, with a variability three and six-tenths times as great.

TABLE 9.—*Range and mean of mean spike densities for the F22 and Dicklow parent rows, and for three groups of F_3 progenies, together with the range and the mean of the mean coefficients of variability (C. V.) for the same three groups*

[Family 35c; grown in 1928 at Logan, Utah]

Strain	Spike-density range	Mean of spike density	C. V. range	Mean of C. V.
	Millimeters	*Milli-meters*	*Per cent*	*Per cent*
F22 parent	23. 8–28. 0	25. 6	10–18	13. 68
Dicklow parent	43. 5–64. 7	50. 3	8–16	10. 54
Homozygous dense	19. 5–33. 2	25. 2	6–22	12. 00
Heterozygous	24. 6–47. 6	34. 7	24–48	38. 15
Homozygous lax	49. 6–62. 4	56. 5	4–16	9. 04

Stewart (*6*) found transgressive segregation in both directions in a cross of Kanred × Sevier wheat. In the present study, with parent rows placed at 10-row intervals in the nursery, a good comparison was possible. The measurements indicate a rather definite tendency toward greater laxness.

It is evident from the material presented that there are three definite groups of progenies when classified according to spike density. When compared with a 1:2:1 ratio a fair agreement was found. Table 10 compares the closeness of fit on a 1:2:1 segregation basis. $X^2 = 2.6297$ and $P = 0.2767$. In 28 cases out of every 100 a worse fit might be expected from chance alone.

TABLE 10.—*Closeness of fit of three groups of F_3 progenies on a basis of 1:2:1 segregation for spike density*

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
Homozygous dense	60	59. 25	0. 75	0. 5625	0. 0095
Heterozygous	128	117. 50	10. 5	110. 2500	. 9383
Homozygous lax	49	59. 25	−10. 25	105. 0625	1. 7732

$X^2 = 2.7210$. $P = 0.2335$.

GLUME COLOR

The F22 parent has a bronze glume of intermediate intensity. The Dicklow parent has a white glume. It has been noted by other workers that bronze is dominant to white. In this family 58 of the

progenies were homozygous for bronze, 110 were heterozygous, and 69 were homozygous for white. Table 11 indicates the number calculated and observed and records X^2 as 2.2405 and $P=0.3331$. No color variations outside the range of the parental variations were observed and both parental types were recovered. The fit indicates that in 33 out of 100 cases, a worse fit might be expected, due to chance alone. This fit is satisfactory in indicating that the inheritance is due to a single major factor difference.

TABLE 11.—*Closeness of fit of three groups of F_3 progenies on a basis of 1 : 2:1 segregation for glume color*

[Family 35c; grown in 1928 at Logan, Utah]

Group	Observed value (O)	Calculated value (C)	C−O	(C−O)²	(C−O)²/C
Homozygous bronze	59.25	58	1.25	1.5625	0.0264
Heterozygous	118.50	110	8.50	72.25	.6097
Homozygous white	59.25	69	−9.75	95.0625	1.6044

$X^2=2.2405$. $P=0.3331$.

CORRELATION STUDIES

In the correlation studies the mean values for the F_3 progenies of each character studied were used.

All possible correlations were made between the four characters for which counts or measurements were taken. The combinations were as follows: (1) Number of culms and culm length; (2) number of culms and awn length; (3) number of culms and spike density; (4) awn length and spike density of all progenies; (5) awn length and culm length; (6) awn length and spike density of homozygous lax and dense progenies; (7) culm length F_2 and F_3; and (8) spike density F_2 and F_3. Table 12 gives a summary of the correlation constants.

TABLE 12.—*Correlation coefficients (r), correlation ratios (η), their respective probable errors (P. E.), and Blakeman's test of linearity for various pairs of plant characters*

[Family 35c; grown in 1928 at Logan, Utah]

Characters correlated	$r\pm$P. E.	$\eta\pm$P. E.	Blakeman's test
Number of culms and culm length	+0.1795±0.029	0.235 ±0.013	1.76
Number of culms and awn length	+ .172 ± .069	.352 ± .049	1.886
Number of culms and spike density	+ .167 ± .0316	.425 ± .028	5.5
Awn length and spike density (all)	+ .142 ± .035	.271 ± .028	1.93
Awn length and culm length	− .055 ± .095	.347 ± .019	6.05
Awn length (fully awned) and spike density	+ .406 ± .094	.6379± .078	3.068
Culm length, 1927, and same, 1928	+ .118 ± .043	.164 ± .0098	1.306
Spike density F_2 and F_3	+ .3785± .0376	.809 ± .048	10.74
Spike density (homozygous dense and lax) F_2 and F_3	+ .383 ± .085		
Spike density (homozygous dense) and number of culms	− .0796± .033		
Spike density (heterozygous) and number of culms	+ .023 ± .178		
Spike density (homozygous lax) and number of culms	+ .269 ± .028		

The correlation coefficient (r) and the probable error (P. E.) were calculated for each pair of correlated characters. The correlation ratio η and Blakeman's test for linearity were calculated for the same characters to determine if the regressions were sensibly linear.

When number of culms and culm length were correlated, no significant results were obtained, as r is only 0.1795 ± 0.029, rather too small to be very significant. (η) is 0.235 ± 0.013 and Blakeman's test is only 1.76.

In the correlation between the number of culms and awn length r is 0.172 ± 0.069 and η is 0.352 ± 0.049, which is seven times its probable error. Blakeman's test is 1.88. Measurements for awn length were taken only on 47 progenies that were homozygous for awns 4.

The correlation coefficient 0.167 ± 0.032 obtained when number of culms and spike density are correlated is not significant. η approaches significance with a figure of 0.425 ± 0.028. This figure and Blakeman's test of 5.5 indicates that the correlation is not linear and that r does not measure all correlation present.

In an effort to determine the cause of this difference found between r and η, three groups were made of the spike density classes and each group correlated with the number of culms as follows: (1) Homozygous-dense spikes and number of culms; (2) heterozygous spikes and number of culms; and (3) homozygous-lax spikes and number of culms. Homozygous-dense spikes and number of culms, gave a low negative correlation coefficient of -0.0796 ± 0.033, which is barely more than twice its probable error. The only correlation in this separation that gave any result approaching significance was where homozygous-lax spike group was correlated with number of culms. The result here was an r of $+0.269 + 0.028$ which is nine times its probable error but is rather small in absolute value.

In comparing the correlation of awn length and spike density of all progenies neither r nor η was significant. The same is true of the correlation awn length and culm length.

The only correlation that gave a positive indication of real value was that which measured awn length and spike density for the fully awned group. In this case r equals 0.406 ± 0.094 and is four and one half times its probable error. η also is large, 0.6379 ± 0.078. Blakeman's test for linearity, 3.088, indicates that the regression is probably linear. It is concluded, therefore, that there is a definite correlation between the length of the awn and density of spike in the fully awned progenies, that is, as the spike becomes less dense the awn becomes longer (*6*).

F_2 culm length was correlated with F_3 culm length of tall plants. It was found that no correlation existed, as neither r nor η was large enough to be even slightly important.

Significant correlations were found between the spike density of F_2 plants and that of F_3 progenies, both for all the progenies and for only the homozygous ones. That there is linear correlation is shown by the fact that $r = +0.378 \pm 0.038$, about ten times its probable error. The large η of 0.809 ± 0.048 which is seventeen times its probable error and together with a Blakeman's test greater than 10 indicates considerable correlation of a nonlinear nature.

Soil Heterogeneity

The Harris method (*3*) was used to study soil heterogeneity. Single rows of each parent variety were grown side by side after every 10 of the F_3 progeny rows, thus insuring systematic distribution. All the contiguous parental and progeny rows received identical treatment within the range of reasonable error.

Correlation studies were made between culm-length measurements of the F22 and the Dicklow parents and also between the spike-density measurements. The results of these correlations are shown in Table 13.

TABLE 13.—*Correlation studies of parent plant characters, F22 and Dicklow, to determine the presence of soil heterogeneity*

[Family 35c; grown in 1928 at Logan, Utah]

Character studied	Correlation coefficient and probable error
Culm length, Dicklow and F22	+0.461±0.111
Spike density, Dicklow and F22	+ .626± .085

In the light of the work of Harris showing that variability which produces a positive significant correlation is due to soil heterogeneity, Table 13 proves that heterogeneity of the soil was measurably noticeable in this field. The culm length correlation gave an r of $+0.461 \pm 0.111$ and that of spike density an r of $+0.626 \pm 0.085$. Both of these figures are significant in indicating the existence of considerable soil heterogeneity, amounting (when solved by the formula $v = 100(1-\sqrt{1-r_2})$, where V = variation in percentage) to about 10 and 20 per cent variability, for culm length and spike density, respectively.

COMPETITION

The ability of one strain to thrive at the expense of the near-by strain is known as competition. One of the plant characters in this cross influenced by competition is number of culms. A correlation was made between the number of culms produced by each parent when grown side by side after each 10 rows of F_3 progenies. These data gave a correlation (r) of -0.557 ± 0.097. With respect to number of culms this significant correlation, five and five-tenths times its probable error, indicates a strong competition between F22 and Dicklow. Experimental results with number of culms were influenced by competition to the extent of about 20 per cent of the variability. Such high environmental variability would tend to hide segregation and may perhaps have done so in this study.

SUMMARY

The cross between F22 (a pure-strain segregate from Dicklow × Sevier) and Dicklow (D3) was made in 1925 at Logan, Utah.

The data were so taken and recorded that the parent and progeny characters could be compared, and in such a manner that correlation studies could be made.

The range of the two parents for culm length was recovered in the progeny, and no segregating groups other than dwarfs were revealed.

The F_2 progenies segregated on a 13 normal : 3 dwarf basis for stature. This was plainly indicated when compared with the 7:6:2:1 ratio expected in the F_3. The Dev./P. E. method was used to separate the classes 13 normal : 3 dwarf, the 3:1, and the 1:3 classes. This calculation was successful for the 1:3 class, but only partly so for the 13:3 and the 3:1. The correct numbers exist in other classes and in these two combined. The evidence as a whole bears out the theory that one dominant dwarf factor and an inhibitor factor were operating in the inheritance of stature in this cross.

The F22 and Dicklow parents were essentially alike in number of culms per plant, and no segregation was observed in the progenies.

There was a single-factor difference in awn-class inheritance. The awn types of both parents were recovered. There was also a segregating awn class.

The mean spike density and the coefficient of variability were calculated for each progeny. One major factor difference was found operating in spike-density inheritance. Three groups were recovered: (1) A homozygous-dense group, like the F22 parent; (2) a heterozygous group segregating for spike density; and (3) a homozygous-lax group which tended to be more lax than the Dicklow parent. The means for 10 rachis internodes were 56.5 ± 1.74 mm. for the F_3 homozygous-lax rows and $50.3 \pm .577$ for the Dicklow parent. The difference is 6.2 ± 1.83, which is 3.4 times the probable error.

A major and one or more minor factor differences are probably operating to bring about the recovery of the F22 parent spike and the greater laxity of the true-breeding lax progenies as compared with the Dicklow spike. The small coefficient of variability determined for the lax class indicates stability of behavior.

Correlation studies were made of the mean values of each character studies in the F_3 progenies.

The correlation between awn length and spike density in the fully awned progenies gave an r of $+0.406 \pm 0.094$, indicating a correlation between these two characters. Another significant correlation was that which compared spike density in the F_2 progenies with that in the F_3, indicating stability of behavior for this character.

Soil heterogeneity was studied by using the coefficient of correlation on parent row characters. These paired parental rows were spaced systematically throughout the plot. The correlations were significantly positive for culm length and spike density and indicated that soil heterogeneity was measurably noticeable in this field.

Competition between varieties was studied by correlating the number of culms produced on the two parental rows growing side by side. This gave a high negative correlation, indicating that considerable competition existed between the contiguous rows.

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AMMONIFICATION OF NITROGENOUS SUBSTANCES BY PURE CULTURES OF MICROORGANISMS[1]

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INTRODUCTION

Ammonification is strictly a biological process, and a necessary step in obtaining the nitrogen from its organic combination before it can be converted into plant food. Much of the early work done on ammonification and the availability of nitrogen in various nitrogenous materials was conducted in liquid media. Lipman and Burgess (*8*),[2] however, used a soil medium and compared in detail 15 pure cultures. They found that the accumulation of ammonia was governed by the specific organism, the source of nitrogen, and the type of soil employed.

The results herein reported were obtained with 19 soil organisms in pure cultures acting on a number of nitrogenous substances in a soil medium. The quantity of ammonia produced is used to compare (1) the ease with which the various compounds are ammonified, (2) the inhibitory effect on ammonification of substances not appreciably ammonified, and (3) the relative efficiency of the 19 organisms in causing the accumulation of ammonia in soil.

TABLE 1.—*Summary of morphological and physiological characteristics of microorganisms* [a] *used in ammonification studies*

Culture No.	Nitrate reduction	Gelatin liquefaction	Starch hydrolysis	Milk peptonization	Indol production	Litmus-milk reaction	Action on glucose	Action on lactose	Action on sucrose	Shape [b]	Motility	Gram reaction	Chromogenesis	Spore formation
10–B	+	+	−	+	+	−	0	0	0	B	+	+	+	+
15–A	+	−	+	−	+	−	+	0	0	C	−	+	+	−
101	(c)	+	−	+	+	−	+	0	0	B	+	+	+	−
107–A	+	+	+	+	+	0	+	0	+	B	+	+	−	+
108–A	−	+	+	+	−	−	0	0	0	B	+	+	−	+
112–C	+	+	+	+	−	−	+	0	0	B	+	+	+	+
119–A	−	+	+	+	−	−	0	0	0	F	−	+	+	+
119–D	−	+	−	+	+	−	0	0	0	F	−	+	+	+
119–F	+	+	+	+	−	−	+	0	0	B	+	+	−	+
119–G	+	+	+	+	−	−	+	0	+	B	−	+	+	+
123–D	+	+	+	+	+	0	+	0	+	B	+	+	−	+
125–C	+	+	+	+	+	+	+	0	+	B	+	+	−	+
203–A	−	+	+	+	−	−	+	0	0	F	−	+	−	+
208–A	+	+	+	+	+	−	+	0	+	B	+	+	+	+
210–B	−	+	+	−	+	0	+	0	0	B	+	+	+	−
210–D	−	+	+	−	−	−	+	0	0	B	+	+	+	−
219–B	−	+	+	+	+	−	0	0	0	F	−	+	+	+
221–B	+	+	+	+	+	−	+	0	0	B	+	+	+	+
223	+	+	+	+	+	−	+	0	+	B	+	+	+	+

[a] All these organisms are aerobic. [b] B=rods; C=cocci; F=filamentous. [c] Undetermined.

[1] Received for publication Nov. 7, 1930; issued June, 1931. Contribution from the Department of Bacteriology, Utah Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 800.

The organisms were isolated from Utah soils by Greaves (*5*, *6*, *7*) during the summer of 1928. A summary of their chief physiological and morphological properties as given by him is tabulated in Table 1. Culture 15–B when used in this work was not a pure culture but a mixture of two organisms.

The following compounds were used as sources of nitrogen: Asparagine, dried blood, peptone, gluten, casein, gelatin, egg albumen, urea, uric acid, hippuric acid, acetanilide, diphenylamine, caffeine, and calcium cyanamid.

METHOD

Common garden soil, high in calcium carbonate, was dried at 115° C. and so ground as to pass a 20-mesh sieve. Fifty-gram portions were used in common tumblers. Dried blood was added at the rate of 2 per cent. All other compounds were added at the rate of 1 per cent. The soil was thoroughly mixed with the nitrogen carriers, 10 c. c. of distilled water added, and the whole sterilized for two hours at 15 pounds.

Urea and asparagine in solution were added to the soil after separate sterilization, the former by heating for 20 minutes in live steam on three consecutive days and the latter by autoclaving at 15 pounds for 15 minutes. The soils were then inoculated with pure cultures of the organisms grown on nutrient agar. Three checks for each organism and three as controls without inoculation were used in each case. A different source of nitrogen was employed in each set. The same 19 organisms were used in all sets, except when otherwise stated.

After four days' incubation at 27° to 30° C. the ammonia accumulated was determined on the whole sample by distilling and titrating. Magnesium oxide was used as the base. The quantity of ammonia accumulated in the sterile checks in each set was subtracted from that in the inoculated samples to correct for the ammonia formed incident to sterilization and other nonbiological factors. For calculations the average of the three check samples agreeing reasonably well was used.

The ammonia produced in the sterilized soil apart from the added source of nitrogen was small. Seventeen cultures were able to accumulate ammonia varying from 0.2 to 1.5 mgm. This measure was made by inoculating 50 gm. of the sterile soil with pure cultures as above, omitting the introduction of another source of nitrogen.

AMMONIFICATION OF ASPARAGINE

Asparagine was readily ammonified by all 19 cultures, but the amount of ammonia accumulated varied over a wide range. Fifty per cent of the organisms showed great activity. Asparagine decomposes in two steps, each yielding one of the two molecules of ammonia it contains. Any organism, therefore, which accumulates more than 50 per cent of the total ammonia brings about, to some extent, both reactions. There were four such organisms. But this need not imply that the activity of those other organisms must be restricted to the first reaction alone. It is quite as logical to suppose that they could cause both reactions but to a less extent, as that they should cause the first step only to occur more completely.

AMMONIFICATION OF PROTEINACEOUS SUBSTANCES

The more complex compounds, including peptone, casein, gluten, gelatin, dried blood, and egg albumen are acted upon variously by bacteria. Simultaneously with deaminization primary splitting of the molecule may be going on and intermediate decomposition products, mostly amines, formed. These changes are brought about by hydrolysis, reduction, and oxidation.

Inasmuch as only 2.5 to 8 per cent (*10, p. 144*) of the total nitrogen of proteins occurs in the free-NH_2 groups and since as high as 34 per cent of the total nitrogen of gluten, 27 per cent of that of casein, and 11 per cent of that of dried blood were ammonified by pure cultures within four days, it is evident that more than one and probably many reactions are being brought about simultaneously by the same organism.

The results obtained from ammonification of asparagine and the proteinaceous substances, and also from urea, uric acid, and hippuric acid, are tabulated in Table 2 and graphically presented in Figure 1.

Peptone was rather easily ammonified by all 19 organisms. This was to have been expected from the fact that the 19 highest ammonifiers of peptone broth were selected for this work from 68 cultures (*5, 6, 7*), which also accounts for the comparative uniformity in the ammonifying property of all the organisms on peptone. This ability to split peptone varying from 11 to 42 per cent of the total nitrogen added, points to the common occurrence of the proteolytic enzymes in all cultures.

Seventeen cultures ammonified gluten, the quantity of ammonia ranging from 6 to 33 mgm. or 7 and 38 per cent of the total nitrogen added. Cultures 119–F and 107–A accumulated more ammonia from gluten than from peptone. Cultures 119–A and 221–B did not ammonify gluten.

Casein was ammonified by 18 cultures, the accumulation of ammonia ranging from 1 to 26 mgm., or from 1.4 to 36 per cent of the total added nitrogen. Casein is the only negative protein broken down by ereptase and is used as a substrate to detect its presence (*13, p. 107*). Therefore erepsin may occur among the caseinases of many of these cultures. It is present in numerous microorganisms (*13, p. 213*).

All 19 cultures accumulated ammonia from nitrogen supplied in the form of gelatin, the quantity ranging from 1 to 20 mgm. The respective ammonification curves for gelatin and peptone when plotted together suggest a correlation between the ammonification of the two substances, the gelatin being, however, considerably lower than the peptone.

Sixteen cultures ammonified egg albumen; three did not. This again illustrates the specificity of microbial action. Cultures 101 and 10–B were fairly active on the other proteins but did not attack this substrate. Culture 101 was the highest ammonifier of asparagine.

Dried blood was ammonified by all cultures, 18 of which accumulated from 1 to 13 mgm. of ammonia, or between 0.7 and 9 per cent of the total added nitrogen. Culture 203–A accumulated 38 mgm., or nearly three times as much as the highest culture below it. This amount is 26 per cent of the total nitrogen added as shown by analysis. In their experimental work on ammonification of dried blood by pure cultures, Lipman and Burgess (*8*) conducted experiments in which

they used three different soils as media with 2 per cent dried blood. After 12 days' incubation at 27°–30° C. they found from 6.01 to 24.36 per cent of the nitrogen ammonified by the cultures in sandy soil, from 1.64 to 6.91 per cent in clay-loam soil, and from 2.24 to 9.41

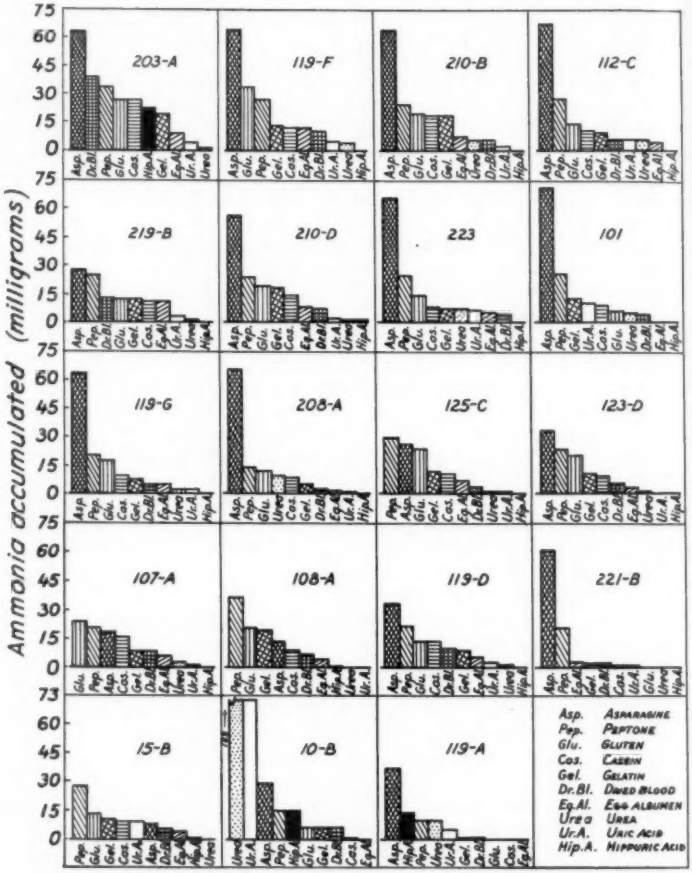

FIGURE 1.—Ammonia accumulated by pure cultures of various organisms in soils containing 10 different sources of nitrogen. The culture number is given in each square

per cent in clay-adobe soil. The decomposition of dried blood is not extremely rapid.

AMMONIFICATION OF UREA, URIC ACID, AND HIPPURIC ACID

Sixteen cultures ammonified urea. Culture 10–B was extremely active. Fifty-nine per cent of the nitrogen added was accumulated by this organism, as contrasted with the other cultures, which accumulated not more then 3.5 per cent, represented by 10 mgm. of ammonia.

It has been reported (*2*) that urea, uric acid, and hippuric acid, in the order named, are increasingly difficult to ammonify. While the total results obtained for the ammonification of urea and uric acid in this work confirm this statement, the margin between the two is very small. In fact, there were more organisms which accumulated more ammonia from uric acid than from urea than the reverse. Table 2 gives the data.

Seventeen cultures ammonified uric acid. Culture 10–B ammonified 36 per cent of the total nitrogen. The other cultures varied from 0.5 to 5 per cent. It is likely that the relationship between the ammonification of uric acid and of urea by culture 10–B is due to the primary formation of urea from uric acid. But, on the other hand, cultures 15–B, 203–A, and 101 represent a group of organisms which accumulate more ammonia from uric acid than from urea.

Hippuric acid, in which the NH is attached only indirectly to the ring was actively ammonified by three cultures and slightly by three others. Culture 203–A accumulated 22 mgm. of ammonia, or 46 per cent of the total nitrogen, and cultures 10–B and 119–A accumulated 15 and 14 mgm., respectively, or about 31 per cent of the total nitrogen.

AMMONIFICATION AND CHEMICAL STRUCTURE

Miyake (*11*) reports that fatty amino compounds ammonify more readily than aromatic amino compounds, that aromatic imino compounds are more difficult to decompose than aromatic amines, and that the nature of the other groups of attachment has no effect on the decomposition of the imino group. Bearing this out, diphenylamine allowed no accumulation of ammonia, while acetanilide having one bond from the NH to the ring was slightly ammonified by five cultures. The ammonia accumulated varied between 0.34 and 1.36 mgm., or 0.6 and 2.2 per cent of the total nitrogen.

TABLE 2.—*Milligrams of ammonia accumulated by pure cultures of various organisms from soil containing 10 different sources of nitrogen*

Culture No.	Milligrams of ammonia accumulated from soil containing—									
	Asparagine	Peptone	Gluten	Casein	Gelatin	Dried blood	Egg albumen	Urea	Uric acid	Hippuric acid
203–A	63	33	26	26	19	38	8	1	4	22
119–F	64	26	33	12	13	10	12	4	5	
210–B	64	24	19	18	18	5	7	5	2	
112–C	67	27	14	10	9	5	4	5	5	
219–B	28	25	12	11	12	13	11	1	3	
210–D	56	23	19	14	18	7	8	1	2	1
223	65	24	14	8	7	4	5	7	6	
101	70	25	6	9	12	4		5	10	
119–G	63	20	17	10	8	5	5	2	2	
208–A	65	14	12	9	5	3	2	10	1	
125–C	26	30	24	11	12	4	7	1	1	
123–D	34	24	21	10	11	6	4	1		
107–A	19	21	24	16	8	8	6	2	1	
108–A	14	37	21	9	20	7	4			1
119–D	34	22	14	14	9	10	5	1	2	
221–B	61	21		1	2	2	3		1	
15–B	8	28	13	9	10	5	4		9	1
10–B	29	15	6	2	6	6		169	73	15
119–A	37	10			1	1		10	5	14

The fact that hippuric acid and other compounds in which the NH group is attached only indirectly to the ring are more easily ammonified than aromatic amines or imines is explained when we know that the initial cleavage is between the ring and the chain attached. Thus with the aid of hystozyme (*13, p. 217*), an enzyme occurring in fungi and bacteria as well as in animal tissue, hippuric acid is split, forming benzoic acid and glycocoll; the latter is then deaminized. Those bacteria which ammonify hippuric acid also ammonify glycocoll (*9, p. 462*).

Caffeine in soil inoculated with cultures 203–A, 107–A, and 10–B, respectively, was not ammonified. The structural relationship between caffeine and uric acid can not be taken to indicate a correlation in the ammonification of the two substances.

Nitrogen supplied in the form of a commercial fertilizer, calcium cyanamid, was not measurably ammonified by any of the 19 cultures within the 4-day incubation period. Four different samples of the cyanamid containing by test from 18.76 to 20.38 per cent nitrogen were run separately with the results just indicated. Furthermore, no ammonia was accumulated, above that in the controls, in 50 gm. of the unsterile garden soil to which 0.5 gm. of calcium cyanamid was added.

A series of reactions has been suggested for the changes undergone by calcium cyanamid in the soil forming successively $Ca(CNNH)_2$, $CNN(CaOH)_2$, and $CNNCa_2CO_3$. The last-named compound hydrolyzes to give ammonia, calcium carbonate, and calcium hydroxide. Because the dicyanamid is hard to decompose and occurs in dilute concentration within the soil, Löhnis doubts the occurrence of the above reactions (*9, p. 590–595*).

Calcium cyanamid can undergo a more direct decomposition in the presence of carbonic acid:

1. $CaCN_2 + H_2CO_3 \longrightarrow CaCO_3 + HNCNH$
2. $HNCNH + H_2O \longrightarrow H_2NCONH_2$
   Urea

Work done by Cowie (*4*) gives good evidence that calcium cyanamid decomposes in suitable sterile soil, forming urea. Added urease then actively ammonifies this product. The fact that in this work such an active urea ammonifier as culture 10–B failed to accumulate ammonia from cyanamid suggests that the soil used was not suitable to catalyze the reaction by which cyanamid is converted into urea. Cowie's work indicates that this is possible since he found some soils, described as heavy and sandy loams, particularly active catalysts to this reaction, while in other soils no urea was formed.

INHIBITORS OF AMMONIFICATION

To test the possible toxicity of acetanilide, diphenylamine, caffeine, and calcium cyanamid, 0.5 gm. of each of these substances was added to 50 gm. of soil containing 1 gm. of dried blood. The soil in the tumblers was inoculated with the six highest ammonifiers of dried blood in pure cultures and incubated four days at 27°–30° C. The data are presented in Table 3.

Diphenylamine totally inhibited a measurable accumulation of ammonia. Furthermore, the quantity of ammonia accumulated by all 19 organisims was greater in sterilized soil than in the presence of diphenylamine.

TABLE 3.—*Inhibiting effect of acetanilide, diphenylamine, caffeine, and calcium cyanamid on ammonification of dried blood (in terms of milligrams of nitrogen accumulated) from soil by various organisms*

Culture No.	Milligrams of ammonia accumulated from soil containing—					
	1 gm. dried blood	1 gm. dried blood+0.5 gm.—				Check on sterile soil
		Acetanilide	Diphenylamine	Caffeine	$CaCN_2$	
203–A	38.08	0.26	----------	0.34	1.71	----------
219–B	12.92	1.96	----------	.34	.34	0.85
119–F	10.54	.80	----------	2.21	----------	.85
119–D	9.69	.43	----------	.34	----------	.34
107–A	7.99	.43	----------	.34	----------	1.02
210–D	7.14	.43	----------	1.70	----------	.85

Ammonia was accumulated in small amounts by all six cultures in the presence of acetanilide and of caffeine. This amounted to between 0.01 and 20 per cent of the ammonia accumulated from dried blood without the addition of these. There was a slight ammonification by two cultures and none by a third where calcium cyanamid had been added to the dried blood. It appears from these limited results that these compounds markedly reduce the formation of ammonia, at least during a short period of incubation.

AMMONIFICATION IN SOIL v. AMMONIFICATION IN LIQUID MEDIA

In order to compare the accumulation of ammonia in soil and in liquid media, the cultures were inoculated into 1 per cent peptone broth and into 1 per cent urea solution, tap water being used for both. Since the soil and broth sets were incubated separately, and, in the case of urea, analyzed by a different method, the results are comparable only in a general way. The data are plotted in Figure 2.

The ammonification of peptone was twice as great in the soil as in the broth and showed greater variation between cultures. The activity of four cultures in broth approached that in the soil. Culture 119–A was actually higher in the broth. Cultures 203–A, 219–B, 108–A, 15–B, and 119–D showed the greatest contrast, the last-named being six times as efficient, measured in terms of ammonia accumulation, in the soil as in the broth.

In studying the same relationship with urea it is seen that the contrast is just as great but on a smaller scale. The same organisms which showed a small margin between the ammonia accumulated in peptone broth and in the soil do not necessarily show the same small margin between liquid and soil media with urea, and vice versa. Culture 101 accumulated more ammonia from urea solution than from urea in the soil. It was culture 119–A which gave analogous results on peptone, while culture 101 showed wide contrast.

THE ORGANISMS COMPARED

A comparison of the ammonifying ability of the 19 organisms can best be made by a study of Figure 1. Since an excess of substrate was always present, the ammonification is measured in milligrams of ammonia and not in percentage of the total nitrogen.

Considering the cultures collectively, 203–A was the highest ammonifier on all substances. It occupied first position in ammonifying dried blood, casein, and hippuric acid and was above the average on all other protein substrates but did not appreciably ammonify urea. This culture is a mold which grows luxuriantly on all media. Cultures 119–F, 210–B, and 112–C were active ammonifiers of asparagine and the proteins. While they compare favorably with culture 203–A on asparagine, they were somewhat lower on peptone and casein and

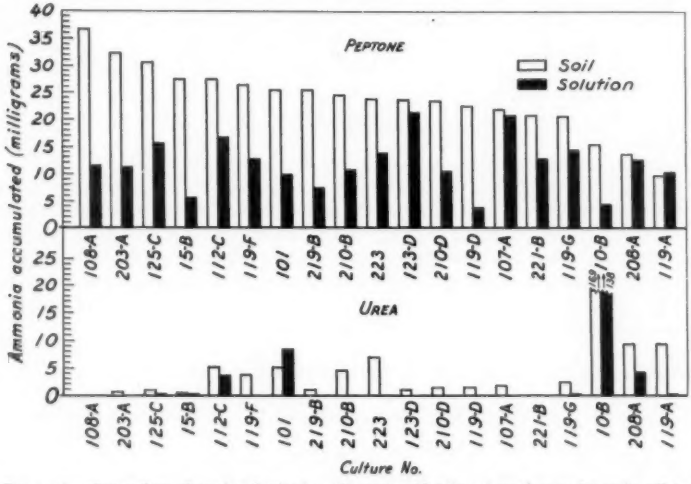

FIGURE 2.—Comparison of results obtained on the ammonification of peptone and urea in soil and in solution by pure cultures of 19 organisms

much lower on dried blood. Culture 119–F was the highest ammonifier of gluten and egg albumen. It ranked second to 203–A in general activity.

Culture 219–B, also a mold, was one of the low ammonifiers of asparagine but was high on dried blood and egg albumen. Its accumulation of ammonia from dried blood, egg albumen, gluten, casein, and gelatin ranged only from 11 to 13 milligrams. The accumulation of ammonia from urea and uric acid by this culture was strictly limited. However, on the whole, its action on all substances was more uniform than that of any other culture.

Culture 101 accumulated no ammonia from egg albumen and little from dried blood, gluten, and casein, but was the highest ammonifier of asparagine. Similarly, culture 221–B showed great activity on asparagine and peptone but very little on all other substrates. But culture 101, unlike 221–B, accumulated 5 milligrams of ammonia from urea and 10 milligrams from uric acid. No. 221–B was inactive on urea and very low on uric acid.

Cultures 107–A, 108–A, and particularly 15–B present an interesting study, for they accumulated more ammonia from peptone, gluten, gelatin (except 107–A), and casein (except 107–A and 108–A) than they did from asparagine. The contrast they present in relation to most of the other organisms is apparent from Figure 1 and from the fact that the average ammonification of asparagine for all cultures was twice that of any other substrate.

Cultures 221–B and 119–A were the lowest ammonifiers of gluten, casein, gelatin, and dried blood. Culture 119–A was also the lowest on peptone and on egg albumen. Interesting enough, it ammonified hippuric acid, uric acid, and urea. Also interesting is the fact that this organism accumulated as much ammonia in peptone broth as in the soil medium, whereas it accumulated none in urea solution despite its activity on urea in the soil.

Culture 10–B is outstanding in its ability to decompose urea and uric acid. Apart from this, it is very comparable to culture 119–A, being low on the proteinaceous materials but also active on hippuric acid. The concentration of ammonia accumulated by this culture in urea media was 0.34 per cent in soil and 0.27 per cent in solution, which is higher than the limit of 0.2 per cent which Bréale (*1*) claims inhibits ammonification. The question of buffer action of the medium might well be raised, but in the liquid medium such action would be strictly limited, as only the soluble salts present in tap water were added in addition to the urea.

Schellmann (*12, p. 22–44*) found two out of five urea decomposers and one out of four uric acid decomposers which ammonified hippuric acid. Of the eight highest ammonifiers of urea here reported, two ammonified hippuric acid; of the eight highest ammonifiers of uric acid three ammonified hippuric acid.

Cultures 203–A, 219–B, 119–A, and 119–D are molds. Three of these are relatively low ammonifiers of asparagine. This may be only accidental.

Culture 223 is the median ammonifier on peptone, gluten, and egg albumen and the mode average in five cases. It caused a total accumulation of 154 mgm. of ammonia on all substrates as compared with an arithmetic mean of 160 mgm. for all cultures. Culture 210–D is another "average" ammonifier.

SUMMARY

Various compounds used as sources of nitrogen were added to soil and inoculated with pure cultures. The order of availability of the compounds in terms of ammonia accumulated in four days by 19 cultures was asparagine, peptone, gluten, urea, casein, gelatin, dried blood, uric acid, egg albumen, hippuric acid, sterile soil, and acetanilide, with zero results for calcium cyanamid, caffeine and diphenylamine. Urea and uric acid owe their relatively high position to the activity of culture 10–B and would otherwise succeed egg albumen.

Calcium cyanamid does not decompose with the formation of urea in the soil here used. The most active urea organisms and even the unsterile soil did not ammonify the cyanamid.

The ammonifying power of soil microorganisms is quite general on amino nitrogen. The nitrogen groups of the purine bases are resistant to the organisms here studied. There are no known criteria by which the ammonifying property of bacteria can be predicted.

Culture 10–B is an outstanding ammonifier of urea and uric acid. This is quite independent of its relatively low ability to ammonify other combinations of nitrogen.

Ammonification in liquid media is lower than in soil, but there is no definite correlation between the two.

Some nitrogenous substances which are themselves not measurably ammonified retard or prevent the accumulation of ammonia from other sources.

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